CLINICAL DIAGNOSIS

A TEXT-BOOK
OF
CLINICAL MICROSCOPY AND CLINICAL CHEMISTRY
FOR MEDICAL STUDENTS, LABORATORY
WORKERS, AND PRACTITIONERS
OF MEDICINE

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To

WILLIAM OSLER, M.D.,

IN GRATEFUL RECOGNITION OF THE MANY KINDNESSES RECEIVED BY A PUPIL AND ASSISTANT, THIS BOOK IS AFFECTIONATELY DEDICATED BY THE AUTHOR

PREFACE

There have, during the past few years, appeared so many and such excellent text-books on clinical diagnosis, the clinical examination of the blood, the urine, or the gastric contents, that to add to this number one which covered the same ground in the same way as they, would seem a thankless undertaking, as well as an unpardonable misuse of energy. It is because the present work tries to cover this same ground in a different way, and one which will, we believe, commend itself to the medical profession, that we venture to offer it for inspection; we refer to the consideration of clinical laboratory work from the clinical rather than from the laboratory point of view.

This book is based on the author's experience as physician in charge of the clinical laboratory, and instructor in medicine, of the Johns Hopkins Hospital and University. He has also had at his disposal all the clinical records of the ward cases for the seventeen years of this hospital's activity.

Our course in clinical microscopy and chemistry extends over the eight months of the student's third year; two afternoons of three hours, and one of one hour, each week; but much of the work is done out of class hours, as inspection of pages 413 and 451 will show. The subjects studied are the clinical examination of the blood, urine, sputum, stomach contents, fæces, and various fluids, as ascitic, pleural, cerebrospinal, cyst contents, etc. In addition to this the student follows cases assigned him in the out-patient department. To those fitted for such work simple problems of research are given. The course is a laboratory one; specimens are provided each of the students. It is needless to say that with the eighty microscopes focussed on eighty specimens of a patient's blood, sputum, etc., the most of the interesting cells or other features will be found. The best were drawn by an artist always within call. The questions discussed in the following pages are for the most part those asked by the students during the class-work. The object of this course is not so much to impart knowledge as to raise the efficiency of the student. It is not a course in chemistry and microscopy, but in these applied to the study of a patient; not in physiology, but in pathology. With the methods of chemical and biological work, with the normal findings, they are already familiar. Chemistry, inorganic and organic, qualitative and quanti-

tative, is required for admission to the school; the normal blood they have studied in the anatomical laboratory; normal urine and gastric contents, in the laboratory of physiological chemistry. We take this knowledge for granted as a foundation for the study of pathological bloods, urines, etc., paying particular attention to the clinical significance of these findings. At the same time the students are required to practise the best methods in every-day use, not only until they understand them, but until they can accurately use them. It is the practical use of a determination or examination which is emphasized. If approximate methods will do, they are used; if accurate methods are necessary, accurate work must be done, whatever the cost in time. To use an approximate method well is far better than to employ a more exact, laborious one poorly; to do approximate work is not always easy and requires practice; to be able to do accurate work well is also required of our students. Practice, experience, an exact knowledge, first of the possibilities in a method, second, and just as important, his own accuracy in the use of that method—these it is the duty of the clinical laboratory to give a student. Above all, he should train his common sense so that, using his eyes, nose, ears, and tongue, he can get results for which another man would apply elaborate methods.

The author has been careful not to include new untried methods, for of these but a small number will last, and a text-book should contain nothing as yet not well tested by friends and foes. It is the introduction of "new methods" which renders some books even dangerous to the man who buys but one.

We do not claim that with this book alone the student can study clinical microscopy. No subject in medicine is broader or requires more reference books, for some of the hardest chemical problems will at times confront him, and to interpret the various artefacts and accidental findings of the microscope would require a vast experience in microscopy, and a knowledge of zoology, botany, and mineralogy as broad as is the realm of science. For who knows what infusoria, what diatom, desmid, or other protophyte, the ovum of what parasite, the wing of what insect, the leg of what fly, the tissue of what plant, the fibre of what meat, the seeds of what berry or fruit, may be found in sputum, stomach contents, urine, or fæces, from the food, tap-water, or the contaminations from dirty vessels, or from the dust of the air? To be wise in the points of differential chemical and microscopical diagnosis is splendid; but to recognize artefacts and extraneous matter, the stumbling-blocks in diagnosis, that is the true test of the chemical laboratory worker, and this ability is gained by wide experience alone.

The function of the clinical laboratory worker is to aid the ward worker. The findings of the former are seldom conclusive, and must be interpreted in the light of the ward findings; especially is this true now that functional diagnosis is the goal. The writer can only give to the reader who has aspirations to be a clinical chemist and microscopist the advice in substance which one of Germany's greatest clinical chemists gave him when the latter regretfully left the little Swiss laboratory which had been such a pleasant home: the clinical chemist must be first a good clinician and second a chemist; he should remember that even from the laboratory point of view his stethescope is of more importance than his microscope, his percussion finger than his whole outfit of chemical apparatus.

In conclusion, we wish to express our indebtedness to Dr. Osler for his encouragement and aid during the progress of this work, and for his hearty co-operation in placing at our disposal the records of the medical wards; and to the assistants and students of this clinic, for whose aid I am very grateful, and who are too many to mention by name except Dr. Thomas R. Boggs, whose suggestions and criticisms have been so valuable.

I take this opportunity to thank the artists who have done much beautiful work for me—Messrs. F. S. Lockwood, Hermann Becker, Max Brödel, and Mrs. Ruth Huntington Brödel, whose excellent half-tone and pen-and-ink drawings must be recognized by the lack of her signature.

Charles P. Emerson.

Johns Hopkins Hospital, 1906.

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INTRODUCTION

The clinical laboratory has two special functions in the medical school,—in it the student learns the application of physical and chemical methods in the study of disease, and in it researches are conducted on the innumerable problems concerning etiology, diagnosis, and treatment. Forming an essential part of the hospital-half of a school, it should be close to the wards and so arranged as to have ample facilities for the students and for the house physicians and others doing special work. It should be in charge of a man resident in the hospital, familiar with the routine of the clinic, and in close daily touch with his chief and with the assistants. The expenses should be shared equally by the hospital and the medical school. Into the details of organization I will not enter, but the director of such a laboratory should, if possible, have assistants thoroughly trained in bacteriology, physiological methods, and physiological chemistry.

In 1896, through the kindness of two ladies, a special clinical laboratory was built for the students of the Johns Hopkins Medical School, which was enlarged two years ago when the new clinical building was erected. On each of the two floors about fifty students are accommodated and there are rooms adjacent for special workers and for the assistants. Dr. Jesse Lazear was at first in charge, and under Dr. Thayer's direction the well-known researches of Macallum and Opie and of Lazear himself on malaria were carried on. In 1900, after Dr. Lazear went to Cuba, we were fortunate enough to have Dr. Charles P. Emerson take charge of the laboratory, and to him the medical school is deeply indebted for the organization of clinical laboratory courses of the most thorough and scientific character.

In medical education the all-important problem is to give a man the knowledge he can use. In our modern system much of the training is rendered ineffective, as it has not been sufficiently prolonged to become part of a man's intellectual or bodily mechanism. A brief course of six weeks on any practical subject is almost useless and in some may be positively dangerous. When possible, an orderly sequence should be followed, so that the work of each year shall supplement that of the preceding. In the seven-year course laid down by the Johns Hopkins University a thorough laboratory training in biology, physics, and chemistry is given before the profes-

sional work begins, so that a man enters the medical school proper with a practical knowledge of scientific methods and of the use of instruments of precision. In his first year of the medical curriculum the courses in histology and physiology and in the second year those in physiology, bacteriology, physiological chemistry, and pathological histology give him an insight into the structure and functions of the body, and he becomes thoroughly familiar with the use of all instruments of precision. In the third and fourth years in the hospital side of his education, for which the previous ones have been a preparation, he must have opportunities to carry on his practical work, and these the clinical laboratory affords. A student who has been interested in the mysteries and mechanism of cardiac rhythm in the physiological course should be able to take the pulse and heart tracings of the first case of mitral disease that he meets in the out-patient department, and the means should be afforded him to pass without a jar from the normal to the abnormal,—without, indeed, appreciating that there is any difference in the method of approaching the problems involved. So too a student should be able at once to attack his first case of diabetes as a problem in carbohydrate metabolism, fully prepared by previous study to approach it on the clinical side.

If the curriculum were not so full, a student could gradually work out for himself, as the patients came under observation, every detail in the application of scientific methods to clinical study, but it is found more convenient to group them together and present in orderly sequence the subjects for study. Concurrently with the systematic instruction in the out-patient department which forms a large part of the work of the third year, a course on microscopical and chemical methods is given, and each man has his own place in the laboratory at which he may work throughout the year. This book is the outcome of the work by Dr. Emerson and his students in this course during the past five years. Not only does it represent the results of a very large number of careful observations made in the laboratory, but an analysis of many important groups of cases in the wards, so that it illustrates the experience of the medical clinic of this hospital so far as it relates to microscopical and chemical methods of diagnosis. The work will be found a comprehensive and trustworthy guide in all the details of laboratory work.

But the aim of a training such as this book implies is to send out into practice men able to give patients the benefit of modern scientific methods in the diagnosis and treatment of disease—men who *use*

the microscope, who examine sputum, and who *use* the stethoscope, and who can do the routine urine and blood work with confidence. The men to study a book of this kind are the young practitioners who are keeping up the practical knowledge obtained in the medical school, and who appreciate a small laboratory as the most valuable stock-in-trade. As a practitioner becomes more and more engaged, he can hand over to an assistant the laboratory side of the work, but it is surprising how much can be done even by the busiest of men if the *will* is there and if the methods have once been thoroughly mastered.

William Osler.

January 30, 1906.

CLINICAL DIAGNOSIS

CHAPTER I

THE SPUTUM

Introduction.—The examination of the sputum is fast becoming a lost art. The discovery of a few specific organisms and the hope of finding more have had as their result the neglect of the study of fresh sputum; the many points which observers of only one generation back carefully noted are now either not looked for, or if they are, are often not seen; a rich nomenclature is forgotten, especially the Latin portion, with the exception of a few terms borrowed from the kitchen. Yet, on the whole, in following a case the careful study of the sputum in the fresh state is very important, and the student who is encouraged, even required, to do this thoroughly, will soon learn that our fathers who never saw a germ could still diagnose and follow cases with an acuity with which he usually does not credit them.

In the examination of fresh sputum the eyes and nose must be trained so that the physician may be able by simple observation to form a judgment concerning the nature of the case, its stage, or the complication which it then presents. For these colors, characteristics, and structures do exist and are to be seen; some are important, and if others are not it is well to know that much; that bacteria in chains are not elastic tissue; a spiral vegetable cell, not a parasite. To search for a parasite is well, but to draw expensive pictures of a few starch granules, confidently expecting that a new one has been discovered, means that the fresh sputum was not systematically studied during student days.

The sputum is, strictly speaking, that substance or mass of substances which is expectorated; in a more common and more limited sense it is that which comes from the respiratory passages, from alveoli to larynx. In its wider sense, considering the variety of sources which may contribute, its importance is great; for besides those from all parts of the respiratory passages it may contain constituents from the œsophagus, nose, mouth, or, through perforation into these, from any neighboring organ.

The presence of any sputum at all is usually considered pathological, and, as a rule, normal persons can raise by coughing either nothing

or very little. Yet the respiratory passages are lined by mucous membrane with active secretory cells, and there is always a certain amount of mucus upon their surface, for those living in a trying climate, or atmosphere loaded with soot, often enough to be expectorated, especially in the morning at which time the cilia have swept to the larynx all the dust, soot, and mucus of the day before.

This morning sputum is small in amount, is expectorated in lumps even the size of a cherry, tough, elastic, gray in color from the coal-dust, and with often a translucency like boiled sago due to myelin. Microscopically, it is of streaked mucus, "the more viscid streaks arising from the goblet cells, the watery from glands" (Panizza), and arranged in lines are epithelial and pus-cells loaded with coal pigment and myelin. In addition are non-nucleated cell-like masses, probably degenerated epithelium, and pus-cells clumped together in balls, and, as a rule, containing no pigment.

When sputum is present in pathological amounts it is raised by coughing, unless in such amounts and with sufficient *vis a tergo* as to flow from the mouth. But there are a certain number of patients who, although there is sufficient sputum for examination, persist in swallowing it, and these must be taught to expectorate. This is particularly true of children, persons of filthy habits, and, of course, partially unconscious patients. The doctor is rewarded for the time spent urging those patients who can to expectorate.

One of Dr. Osler's assistants created some amusement by assiduously sitting by the bedside of a case with suspicious lung signs begging her to expectorate. At last he got a very little, but it contained tubercle bacilli, and the hospital record for early diagnosis of the pneumonic type of pulmonary tuberculosis was broken.

In some cases the swallowed sputum is obtained by washing out the stomach. In children the stools must be examined. In the case of young children the point mentioned by Findlay is valuable; the finger, covered with gauze, is put into the child's throat to stimulate coughing, and the sputum wiped out with this finger before the child can swallow it.

The patient must be carefully taught to avoid expectorating saliva, nasal and pharyngeal mucus, etc., into the cup.

Amount.—Some general idea of the quantity expectorated is always necessary. The accurate measurement of the twenty-four hour amount, though rarely valuable, is often of aid in following a case.

In some cases, although rare, with severe cough, the sputum is so small in amount and so viscid that there is practically none obtained. Such are cases of "dry" bronchitis, diffuse bronchitis, beginning tuberculosis, rare cases of lobar pneumonia, and of caseous pneumonia. Very much is present in certain cases of chronic bronchitis, advanced

tuberculosis with large cavities, bronchiectasis, gangrene of the lung, œdema; in hemorrhage, perforating pleural exudate, and lung abscess the blood or pus may pour from the mouth, or even drown the patient. After too rapidly or thoroughly tapping the chest the amount of albuminous sputum may be great.

The clinical chemist engaged in metabolism experiments must remember to take an abundant sputum into account, since the amount of nitrogen thus eliminated may be even 5 per cent. of the total output.

Consistency.—Generally speaking, this varies inversely as the amount, except in pneumonia, in which case, although abundant, it will not drop from the inverted cup. In true bronchial asthma during the first of the attack, acute bronchitis, and pertussis it may be very tenacious. As a rule, this characteristic is due to mucin. The explanation in the case of pneumonic sputum with little mucin is not so easy, since the water-content is so high. It is ascribed to the nucleins present in abundance, and these in alkaline medium. On the contrary, when there is little mucus and much water, as in œdema of the lungs, or pus poured from a bronchial tree denuded of its mucous membrane, it is very watery.

Reaction.—When fresh, the sputum is alkaline in reaction. That which has stood some time in the cup, or sputum which has stagnated in the body, is acid.

Character.—Mucoid sputum is glairy, transparent, and tenacious. If acetic acid be added, it becomes cloudy (due to the mucin). Such sputum is seen in acute bronchitis, pertussis, and early in asthma.

A mucopurulent sputum is one in which there are enough pus-cells to change the color macroscopically. There is every gradation from one almost mucoid to pure pus. Small amounts of pus give a whitish color, more, a yellow or yellowish-green, the exact reason of which is uncertain. There are two varieties of mucopurulent sputum; in the one the body of the sputum is pure mucus, and in it are suspended streaks and dots of pus. In the other obtains a homogeneous mixture of mucus and pus, yet not enough of the latter to merit the use of the term purulent. Such sputum is only slightly opaque.

Purulent sputum is said to differ from pure pus only in the tenacity due to mucus; but this distinction is artificial, since in broncho-blennorrhœa there is little normal mucous membrane left. Pure pus may constitute the sputum in ruptured empyema, abscess of lung, rupture of an abscess of a neighboring organ through the lung, trachea, œsophagus, or nasal passages.

Serous sputum is colorless, and very frothy from the high percentage of albumin. It is seen in œdema of the lung, perforating serous pleurisy, and in rare cases following thoracentesis.

Color.—Bloody sputum may be almost pure blood, or gain the

name from its slight blood-staining. It is found after trauma, hemorrhagic infarction of the lung, gangrene, early in acute lobar and also caseous pneumonia, pulmonary tuberculosis, tumors of the lung, intense chronic passive congestion, and "weeping" aneurism. As a rule, the blood is mixed with mucus, hence is covered by a frothy layer. The blood may be due to diapedesis or to the rupture of a vessel. Hemorrhagic sputum is in the former case a sign of severe inflammation of the lung, but of no one disease (see page 78).

Sputa colored by the *derivatives of hæmoglobin* may be of almost any color. Formerly it was taught that varying amounts of blood could explain this variety of colors, but Traube proved that unchanged blood-cells could give only a red color or reddish tint. Blood-cells retained in the lung, either in alveoli, bronchi, or tissue, soon lose their hæmoglobin, and the various oxidation products of this can give that wide range of color seen, for instance, in a subcutaneous bruise; various shades of red, brown, green, orange, yellow, chocolate. A few cells may be found, but they are pale and swollen. The best example is the typical rusty sputum of pneumonia, the color of which is due to an unknown derivative of hæmoglobin, but the sputum may be any shade of green or yellow, red, or brown. After hemorrhage into the lung-tissue, cavities, or alveoli, and the diapedesis occurring in chronic passive congestion due, for instance, to mitral disease, there may be sufficient epithelial cells loaded with granules of changed blood pigment to give a characteristic light brown color to the sputum. In destructive processes there is sometimes sufficient hæmatoidin present to give the sputum a dirty brown color. Such is the case in gangrene, abscess, infarction, and chronic passive congestion. These crystals may literally fill the sputum.

Bile pigments are present in the sputum in case a liver abscess perforates through the lung or the person is jaundiced, but except in icterus the term "jaundiced" should not be used. It is granted that chemically the difference between the pigments of bile-stained and similarly appearing sputum with oxidized hæmoglobin is nil, and this may be true of hæmatoidin, but, clinically, the difference between these sputa is too important to neglect.

As GREEN SPUTA are of such importance they should be grouped together. When a patient is jaundiced, the pure mucoid sputum of a bronchitis, for example, may be of a fine grass-green color. In such cases it is the oxidized bile pigment which gives the tint. But when no jaundice is present, exactly the same color (due to the same pigment, perhaps, but with a very different significance) is sometimes seen. This occurs in ordinary croupous pneumonia during lysis, in which case the pigment is oxidized before expectorated, a process for which there is hardly time in an ordinarily sharp attack; pneumonia

ending in abscess; and subacute caseous pneumonia. It is interesting that Traube,[1] who first called attention to these green sputa, gives illustrations of caseous pneumonia alone. In the five cases he cited it was an early feature in three, lasting two to five days, in two for two weeks; it did not remain green in any case till death; in one case the onset of a fresh involvement was accompanied by a return to rusty sputum. In some cases of certain green tumors (chloroma) of the lung there is green sputum; finally, certain chromogenic bacteria may explain the color.

Among the other appearances of the sputum may be mentioned the black sputum of ANTHRACOSIS, found especially in coal-miners, but to a lesser degree in all city residents. The sputum is stained by the particles of inhaled coal-dust. Some insist that it is only the dust *en route* to the lung which is gathered by the phagocytic cells and expectorated, hence should the person move to a locality without that dust the sputum would soon be free, no matter how loaded the lymphatic channels of the lung might be, and a return or continuance of the pigmented sputum would mean the presence of a destructive process. Yet some coal-miners without any suspicion of tuberculosis have black sputum for years after they have given up that occupation (Osler). Among the other pneumoconioses are SIDEROSIS, in which the sputum is stained red with the ferric oxide inhaled by mirror polishers; workers in brass and bronze have a sputum stained by metal: those inhaling mineral dust suffer from CHALICOSIS, "stone-cutters' phthisis," "grinders' rot," and expectorate much of that dust; this condition is common among the stone-hewers who work with sandstone, and hence cases are frequent in the Strassburg clinic. The chest is retracted, since the large amount of dust leads to malnutrition of the lung. Infection of the lung is easy, local gangrene even with pneumothorax may result. They may give a long history of hæmoptysis without tuberculosis, but sooner or later all become tuberculous. Workers with ultramarine blue or methylene blue, or similar dye-powders, have deeply stained sputum. Millers and bakers expectorate doughy masses, and the sputum of cotton-mill operatives is often full of that fibre.

The observer must not be deceived by various vegetable or animal fibres, or by food or drink mixed with the sputum. Milk, eggs, wines, coffee, chocolate, tobacco, licorice, and various medicines can confuse one.

Finally, chromogenic bacteria may change much the appearance of sputum, especially in summer,—*e.g.*, bacillus virescens, pyocyaneus, and many others. Sometimes in a "sputum cup ward infection" the cups in a series may show the presence of these organisms. The sputum when expectorated will of course not be thus colored.

[1] Gesam. Beitr., ii. p. 699, 1871.

AIR is present in the sputum in various amounts and in bubbles of various sizes. From the size of the air-bubbles can in a general way be determined the size of the bronchi in which the sputum was formed, and the effort required to expel it. Sputum from cavities and large bronchi contains no air, and hence sinks in water. This "sputum fundum petens" was formerly given an overrated diagnostic value, since it was supposed to indicate a cavity.

The layer formation of sputum is of value. In certain conditions, especially bronchorrhœa, bronchiectasis, putrid bronchitis, and gangrene of the lung, the sputum is abundant, and in a tall jar will separate into three layers,—an upper of frothy mucus, a lower of morphological elements, pus, tissue shreds, detritus; and a middle of the pus serum, usually an opaque watery fluid. Often a fourth layer just under the mucus consists of the material of the sediment and hangs in long shreds down through the pus-serum.

Odor.—Ordinarily the sputum when fresh has almost no odor. Sputum allowed to stand, or that which has stagnated in the body, soon gains, or has when expectorated, a very positive odor; that of tuberculosis and bronchiectasis is heavy, sweet, and penetrating; that of a perforating empyema is said to resemble old cheese; that of putrid bronchitis and many cases of bronchiectasis is fetid; that of gangrene is usually the worst of all. The odor of the breath has some importance, especially in tuberculosis, for it may be fouler than the sputum in the cup, perhaps owing to the fact that the warm sputum in the body scents the air more than when cold, in which case it may be odorless. Some have claimed to have diagnosed small cavities by this sign before they could have been discovered by physical examination.

Macroscopic Constituents.—SMALL MASSES OF PUS are common, whose size indicates, to a certain degree, the size of the bronchi from which they arise.

FRAGMENTS OF NECROTIC TISSUE occur, sometimes large in abscess and gangrene of the lung, but small in tuberculosis in which disease large masses are rare except perhaps from the wall of a cavity around which is such active proliferation of connective tissue that the necrotic tissue is dissecting free. The great majority of the fragments are almost at the limit of gross vision. The fragments from an abscess are permeated by pus-cells, hence are yellow in color; those from other conditions are dark from changed blood, while the smaller ones are black, often from coal pigment. The recognition of even the smallest is important, since in them one has the best chance of finding elastic tissue.

If the sputum be squeezed out between two plates, these small fragments can be seen as yellowish, often pigmented threads, for the most part just on the limit of vision while some are even 2 cm. long; or

as masses from those very minute to those the size of a pea. The search is much facilitated by a small hand-lens. They are found in the greatest numbers in the nummular masses from a tuberculous cavity. Necrotic fragments of cartilage from tuberculous ulcers of larynx, trachea, or bronchi are sometimes found. Tumor fragments should be looked for.

Dittrich's plugs are bodies of considerable interest. They are sausage-shaped casts of bronchi, varying in size from very small to those the size of a bean, but the majority from that of a millet- to a mustard-seed. The smaller are of an opaque yellowish-white, the larger of a dirty gray color. If crushed between the fingers they are found to have a horrible stinking odor. Microscopically, they consist for the most part of zooglia of bacteria, fatty acid crystals, fat droplets, and cell detritus. Few cells are contained, except in some a few leucocytes indicating perhaps that the plug is fresh. Pigment granules, fragmented red corpuscles, hæmatoidin crystals, flagellates, and a leptothrix taking a fine blue with iodine solution and not yet well studied, have been found. The fatty acid crystals of the larger plugs are long and curved, while those of the shorter are fine needles. These plugs occur in any putrid disease, especially putrid bronchitis and bronchiectasis, in which case they are especially large. How these are formed we do not know (Hoffmann). Similar plugs are derived from the crypts of the normal tonsils, and especially in case of follicular tonsillitis. These are of beech-nut shape.

Curschmann's spirals are perhaps the most beautiful structures found in the sputum. They occur at some time in practically every case of true bronchial asthma, and have been reported present in acute

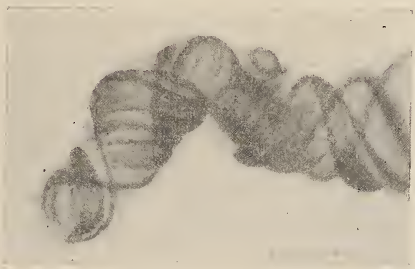


Fig. 1.—A spiral thread of mucus from the sputum. × 5.

bronchitis, acute lobar pneumonia, chronic pulmonary tuberculosis, and in rare interesting cases which seem to stand between bronchial asthma and fibrinous bronchitis, in which are expectorated small fibrinous casts with a few typical spirals directly continuous with the tips of their branches. Curschmann considered the spirals due to a bronchiolitis exudativa. (For a description of these spirals, see page 62.) In some

sputa coarse strands of mucus and pus may be twisted into a spiral shape (see Fig. 1).

Fibrinous Structures.—Under this head we include all structures ordinarily thus termed, although in some the presence of fibrin is rather doubtful.

The pseudomembranous casts of *diphtheria* are sometimes present in the sputum. If from the throat, larynx, or trachea, they are in unformed masses, but if from the bronchi, may form arborescent casts, from the size of which may easily be judged the extent of the process. These are whitish in color and contain many epithelial cells. In *pneumonia* casts of smaller bronchi are found very often if one takes the trouble to search for them (see page 58). These are more brownish or reddish, and contain blood and many leucocytes. The most beautiful casts occur in the *chronic idiopathic fibrinous bronchitis* (see page 70). *Acute fibrinous bronchitis* accompanies various fevers, typhoid, erysipelas, measles, smallpox, scarlet fever, acute articular rheumatism, also exophthalmic goitre, pulmonary tuberculosis, mitral disease; in the rare albuminous expectoration after thoracentesis, and after the inhalation of irritating vapors and gases, similar casts have been found. Bettmann [2] gives a good review of the subject.

In addition to well-formed arborescent casts occur unformed masses of similar nature, evidently also from the bronchi. These were perhaps expectorated before a definite cast could be formed.

That much of this material is fibrin is very doubtful. The tests generally applied are; the physical properties of the mass (color, toughness, etc.), the fact that it swells and clears in acetic acid (which precipitates mucin), and the rapid effervescence on the addition of hydrogen peroxide. Hirschkowitz, in one from a case of tuberculosis, found only fibrin present.

Casts formed of the *mycelium of fungi* have been found. In Osler's case the small cast consisted of the mycelium of some form of the aspergillus. Casts due to a similar parasite were expectorated for years by the case reported by Devillers and Renon.[3]

Lung Stones.—This name is applied to almost anything having the appearance or consistency of a stone. Theoretically, they could be cartilaginous, osseous, or calcareous, but to the last alone is the term strictly applicable.

Enchondromata and *osteomata* of the bronchi and lungs are found at autopsy, but among Poulalion's cases (Thèse, Paris, 1891) we could find mention of none in which they were expectorated. Neither do we know of any case in which the stone has arisen in a calcified infarct, nodule of bronchopneumonia, miliary abscess, pseudotubercle of

[2] Am. Jour. Med. Sci., February, 1902.
[3] La Presse Med., 1899.

actinomycosis, cladothrix, or moulds, nor from the calcified wall or contents of a cyst or tumor, although at autopsy such concretions are found.

Fränkel mentions one case in which the stone was a fragment of the bronchial cartilage which had become calcified and then dissected free, and Hoffmann one of a calcified blood-clot.

Lung stones are in the great majority of cases due to tuberculosis.

The *Bronchioliths* are formed by the deposit of salts in the stagnated contents of a bronchus or bronchiectatic cavity. They may be from smaller or larger bronchi. One would expect them to be arborescent, but for the most part they are irregular, jagged, from the size of a millet-seed to a nut. They may be chalky or stony hard. As a rule, they are single or at most two or three in number, but sometimes several hundreds. Poulalion suspects that these great numbers are fragments of larger stones. Some " resembling coral, finely ramified, and very hard" have been described. In one case the stone weighed 0.47 gm. and had ten or twelve branches. In Atlee's case [4] the stone was three-quarters of an inch long and one-quarter of an inch wide at the larger end.

Pneumoliths may be calcified caseous areas, which, treated as foreign bodies, ulcerate into a bronchus, or the contents of a closed cavity which become impregnated with lime salts and then set free. Another source, perhaps a common one especially in those cases in which the lung parenchyma was normal, are the calcified bronchial lymph glands. Of the pneumoliths, there are two distinct varieties,—the cretaceous, of chalky consistency, and the calcareous, small as a rule, hard, and with a rough, rounded surface. Their size varies from that of a millet-seed to a pigeon's egg.

Chemically lung stones consist of calcium and magnesium as bases with carbonic, phosphoric, and sulphuric acids, also traces of ferric oxide and other metals. Their composition varies; in some, one or another salt predominating in a large mixture, while others seem composed of but one calcium salt.[5] Unless branched, or a soft cretaceous stone with considerable of the tissue structure remaining, it is quite impossible to tell its source. In some cases they are expectorated in great numbers, even 200 and, in one case, 500. Since their occurrence is so rare, and in those cases in which they do occur they are so marked a feature, the condition formerly termed " pseudophthisis calculosa," Hoffmann and others consider it necessary to assume a constitutional abnormality, an increased excretion of lime salts through the lungs. In these cases the " hæmoptysis calculosa" is often a feature of the

[4] Am. Jour. Med. Sci., vol. cxxii., 1901.

[5] See Stern, Deutsch. med. Wochnschr., 1904, No. 39; Carlyon, Brit. Med. Jour., 1890, ii. p. 1474.

"bronchial colic" accompanying the expulsion of the stone, and is due to trauma of the mucosa; while usually not abundant it may be extreme. Abscess, gangrene, or pneumothorax may result from the presence of these stones. Some concretions have a foreign body as a nucleus, a cherry-stone or a grain of wheat. In others the lung-tissue impregnated with the salts remains, and when decalcified the structure of the lung, even with some few remaining nuclei, may be seen in the sections, and the tubercle bacilli demonstrated.

Among FOREIGN BODIES expectorated may be mentioned teeth, cherry-stones, and coins.

Fragments of the wall of echinococcus cysts or the daughter cysts themselves may be expectorated.

FIG. 2.—Extraneous matter common in the sputum. Threads of, A, linen; B, silk; C, cotton; D, wool; E, starch granules; F, guard cells from a lettuce leaf; G, squamous epithelium from tongue, with bacteria attached; H, tobacco, showing the surface of the leaf, the large cells stored with oil, and a spine from the surface. × 200.

Microscopical Examination.—The microscopical examination of the fresh sputum is easy, valuable often, but very much neglected. The technique is of course simple. A little sputum is spread upon a plate, the base of which is half black, half white, and the interesting particles chosen and squeezed between the cover glass and the slide. Thin specimens are essential. The first point of importance is that the observer recognize at a glance the EXTRANEOUS STRUCTURES, and these are many in number. Among them may be mentioned almost any of the food-stuffs, but particularly fragments of bread, bits of orange-pulp or other fruits, drops of milk, portions of jams and preserves, the skin of fruits, portions of tobacco, etc., portions of meat in which is elastic tissue, and fragments of vegetable leaves. In addition, it is important to recognize various threads, particularly fibres of linen, cotton, wool, and silk, and small fragments of paper (see Fig. 2).

Pus-cells.—Thin smears of sputum may be treated like blood smears and stained with the same stains, especially the methylene blue-eosin mixtures.

The pus-cells are usually polymorphonuclear neutrophiles. They are spherical in shape, of from 7 to 10 microns in diameter. These granular cells are often filled also with fat globules or pigment granules; in some glycogen may be demonstrated. In asthma the eosinophilic cells usually predominate, and one may search long for any other form. The diagnostic value of these cells in tuberculosis has been much disputed; there is a form of bronchitis which has been known as "eosinophilic bronchitis," so many such cells are present. Hilderbrandt[6] holds that they are of such common occurrence that their presence speaks neither in favor of asthma nor against tuberculosis.

The various epithelial cells are important. Since these come from several sources various forms may be expected and their origin should be recognized. *Pavement epithelium* may come from the mouth, the pharynx, and the respiratory tract as low as the vocal cords. It is a very valuable lesson for the student to scrape from the surface of the tongue by means of a cover-glass a little of the superficial epithelium and study the masses of epithelium covering the villi, to which are attached large zooglia of bacteria. *Cylindrical epithelium* may come from the nose or bronchial tree. While the cylindrical epithelium cells from the trachea and bronchi are both goblet and ciliated in the sputum, the majority soon lose their original shape and can merely be recognized as cylindrical cells. These occur early in bronchial catarrh, and later are replaced by pus-cells. It is seldom that cells which are actually ciliated are seen in the sputum except in asthma, ulcerative processes, and recent bronchitis. In a recent case of asthma small clumps and a rather large sheet of cylindrical epithelium were found.

The alveolar epithelial cells are important because of their considerable number in nearly every sputum examined, even of normal persons, since they are desquamated as from all epithelial surfaces; and because of the large variety of forms which they may assume, some of which it is very difficult to recognize. Every observer is pretty certain to be deceived once or oftener by these cells. In general, they may be said to be from four to five times the size of a leucocyte, oval, with a coarsely granular protoplasm and one or more large, oval, vesicular nuclei. They are found in normal sputa, as well as in almost every other condition. Their large number in bronchitis would indicate some intimate pathological relation between the bronchi and the alveoli. They are most numerous in inflammatory processes of the lung, and especially in tuberculosis, where they may fill the alveoli, although retained here and hence do not appear in the sputum. These cells are

[6] Münch. med. Wochnschr., 1904, No. 3.

amœboid on the warm stage, and would seem to be important phagocytes in the lung. Formerly their origin was disputed, but now it is agreed that they arise in the alveoli.[7] The inclusions of these cells are many. *Coal pigment* (see Fig. 3, *a*) explains the black granules present in all morning sputa of even normal persons, representing the inhaled dust of the day before. The origin of these black granules was long in dispute, until in one cell was found a granule in which the structure of wood was unmistakable. This patient had evidently inhaled charcoal dust. These pigmented cells are often present in large numbers, and give the sputum a smoky, grayish, or dirty green color. When present in large numbers in certain diseased conditions, the old term "phthisis melanotica" was applied. Some of these alveolar cells are filled with smaller or larger, very refractile, round, *fat globules* (see Fig. 3, *i*). *Myelin globules* are irregular in shape, not perfectly spherical, often presenting concentric lines, with very little refractivity, and of a dull greenish or blue appearance. They may be few in number or fine, filling the cell; or one may occupy the most of the cell (see Fig. 3, *h*). Some think these represent the products of degenerated protoplasm; others that they are a normal secretion of the bronchial mucosa which these phagocytes accumulate;[8] others that they arise from the goblet-cells, but the absence of the free granules in the nasal and pharyngeal mucus speaks against this. When large groups of these cells are present, as in normal morning sputa, in some cases of bronchitis, acute or chronic influenza, and sometimes pneumonia during resolution, and especially the "desquamatory catarrhal pneumonia," the sputum contains small lumps markedly resembling boiled sago, hence the name "sago granules." *Free myelin* may occur in large amounts as sharply defined, palely refractive drops of very different sizes and peculiar shapes (see Fig. 3, *j*). It was to these, from their resemblance to the myelin globules of nerve-tissue, that Virchow gave the name "myelin drops." While the term "myelin" carries with it no hint of the chemical nature of the droplets which are seen in the sputum, urine, stools, or nervous tissue, since substances of very various nature—fatty acids, oils, neutral fats—can give droplets of just this appearance (Liebreich), F. Müller and his students think that those of the sputum consist chiefly of protagon, with some cholesterin and lecithin. They swell somewhat in water, are not destroyed at 100° C., are stained yellow by iodine, stain poorly with aniline dyes, do not turn black with osmic acid, are easily soluble in alcohol, slightly in ether and chloroform. The amount of myelin in the morning sputum may be so great that it seems to surpass that of the mucus. To a certain extent the excre-

[7] See Hoffmann, Nothnagel's System, Die Krank. der Bronchien.

[8] See Schmidt, Berl. klin. Wochenschr., January 24, 1897.

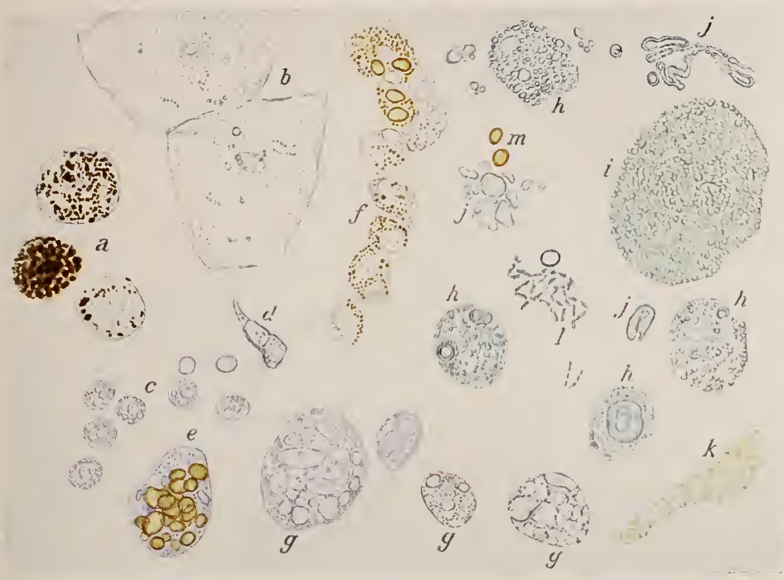


FIG. 3.—Cells in the sputum. *a*, alveolar epithelium cells containing coal-dust; *b*, squamous epithelium cell; *d*, cylindrical epithelial cell; *e* and *f*, Herzfehlerzellen; *g*, cells showing a peculiar degeneration; *h*, those with myelin droplets; *i*, one full of fat droplets; *j*, free myelin; *k*, red blood-cells; *l*, bacteria; *m*, free blood pigment. × 400.

tion of these two bodies runs parallel. It presents a large variety of shapes; concentric spheres or club-shaped masses, small globules or by confluence of small globules, larger drops (see Fig. 3, *j*). Their number and size increase much on standing. The alveolar cells containing *derivatives of hæmoglobin* are of particular interest. The hæmoglobin, certainly derived from the red blood-cells of the sputum, may be present in amorphous granules or scales of a brownish color, or hæmatoid crystals. "Herzfehlerzellen" (see Fig. 3, *f*) is the name given to these cells filled with a golden yellow pigment, providing they occur in large numbers, and over a long period of time; only then have they any diagnostic importance. The granules are sometimes small but often large. They have a translucent appearance, not being opaque, and certain cells seem to be diffusely stained. Since these granules are not opaque and deeply colored, but seem only tinged yellow, the student at first is disappointed in their appearance. In chronic passive congestion, especially that due to mitral disease, these cells may give a gross color to the sputum, the entire mass being of a rusty color; or, what is more common, they are clustered into dots and streaks of a reddish-brown color in a white mucous background. They occur also in all other conditions in which red blood-cells escape into the alveoli, hence in pneumonia, infarction of the lung, and after pulmonary hemorrhage.

The red blood-cells (see Fig. 3, *k*) in the sputum are often well preserved, yet not always, as is seen by the masses of amorphous hæmoglobin and by the inclusions of the alveolar cells. They are crowded into lines and masses, allowing nothing of their shape to be seen, and recognizable only by their color. They are sometimes squeezed out into long threads. In judging the importance of blood in the sputum, however, even macroscopically, it is well to bear in mind the numerous sources where it may have arisen; for instance, the nose, the mouth, and the pharynx.

Elastic tissue is a most important body. Formerly its presence was of greater importance, since before the discovery of the tubercle bacillus this was the best evidence of consumption. Even now it is of considerable value, since its presence indicates certainly destruction of the lung, and in some cases it is found before the tubercle bacilli, but perhaps not before they are present. The masses of elastic tissue are usually almost on the limit of vision with the unaided eye. This is particularly true in tuberculosis, in which case molecular disintegration is the rule, although in some cases only single fibres may be found. The Sir Andrew Clark method is the one which Dr. Osler recommends, since by its use the various methods of destroying other tissue, such as boiling with sodium hydroxide, etc., may be dispensed with. For this two glass plates are used,—the one about fourteen and the other six

inches square. The sputum is poured on the larger plate, pressed out by the smaller. The plate should rest on a dark background. With a small hand-lens fragments of tissue may be easily selected, and then

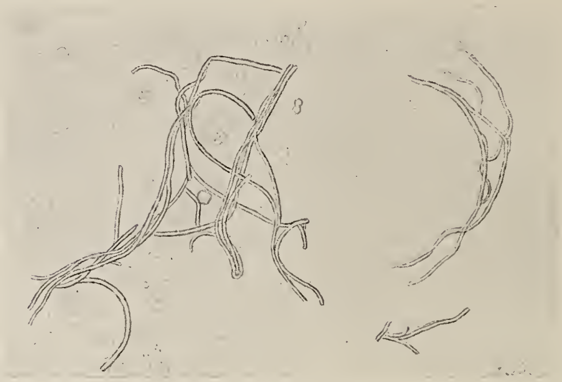


FIG. 4.—Elastic tissue from lung. × 400.

after sliding the upper glass away from them they can be picked up with a needle. These appear as small grayish-yellow spots. In some cases it is not necessary to remove them for inspection with the higher power, since a small pocket-lens will be sufficient. Others prefer Petri's dishes, or wooden boxes with black base, or crockery plates with the base half black, half white. One must be very careful not alone to sterilize this glassware, but to wash it well in chemicals which will

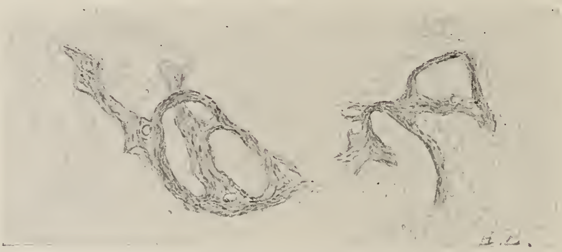


FIG. 5.—Elastic tissue from lung showing alveolar arrangement. × 50.

destroy all organic matter (a saturated potassium bichromate solution in concentrated sulphuric acid is recommended), else there is chance that the bacilli found may not be from the sputum in question. Particles of food will confuse the beginner. Under the cover-glass small fragments of elastic tissue may be found with the low power of the microscope. Larger fragments of lung-tissue are sometimes present. When no fragments are found in this way, a search with the higher power must be made for single fibres of elastic tissue, in which case it is well to select the grayish masses of sputum from the

tuberculous cavity or the particles of grass-green or slightly rusty sputa which are present in a subacute caseous pneumonia. The fresh specimens must be very thin and the cover well pressed down, or the fibres may be overlooked.

The elastic tissue from the lung may be present in three arrangements: most important is the alveolar form, in which case the fibres preserve the outline of one or several alveoli (see Figs. 4, 5), and the fibres are long and branching; from the bronchi, in which case the fibres are single or in small groups, fragmented and less curled than in the former, Dr. Osler considering that the most characteristic picture is that of two or three long narrow fibres clustered closely together in an elongated net-work; from the arteries may arise a distinct sheeting. Fragments containing a coarse net-work of short interwoven fibres are seen from ulcers of the larynx.

When the elastic tissue is very small in amount it is customary to destroy all other tissue by means of potassium hydrate, but if the above Clark method be carefully used this method is not necessary. Ten cc. of sputum are mixed with an equal amount of 5 to 10 per cent. KOH or NaOH; the mixture is then boiled in a porcelain dish until the mass is homogeneous. About four volumes of water are then added, the entire mass shaken up and then centrifugalized. Nothing is left but the elastic tissue. The fibres, however, have lost their characteristic appearance, being now paler and swollen.

The fibres of elastic tissue (see Fig. 4) even when single should be recognized. They are characterized by their intense refractivity, their waxy outline, their sharp edges, their uniform diameter, and their curling ends. They often branch. They are insoluble in ether, potassium hydroxide, and on warming the slide. Pressure does not cause any varicosities. Their appearance is very characteristic with the low power, perhaps more so than with the higher, although the latter should always confirm the former. In the thin specimen they will stand out as very distinct, coarse, sharp, blackish fibres. It is necessary to exclude fibrous tissue, fatty acid crystals (see Fig. 6), bacteria, and vegetable cells and fibres. The fibrous tissue fibres are very different in appearance, being present in bundles of fine wavy lines without the coarse black refractive appearance of elastic tissue. (For the fatty acid crystals, see page 33.) The chains of bacteria are very confusing, especially certain leptothrix forms (see Fig. 7). These are found in the fresh sputum, but especially that which has stood for some time. The long chains will sometimes present a beautiful interlaced net-work and sometimes simulate closely the framework of an alveolus. Under the high power, however, it will be seen that these fibres are chains of bacilli. They differ also in size and in refractivity and in the absence of the wavy outline. They are also much more crowded

in the field than is elastic tissue. Vegetable cells and fibres are much coarser and should not confuse. The elastic tissue from food is often

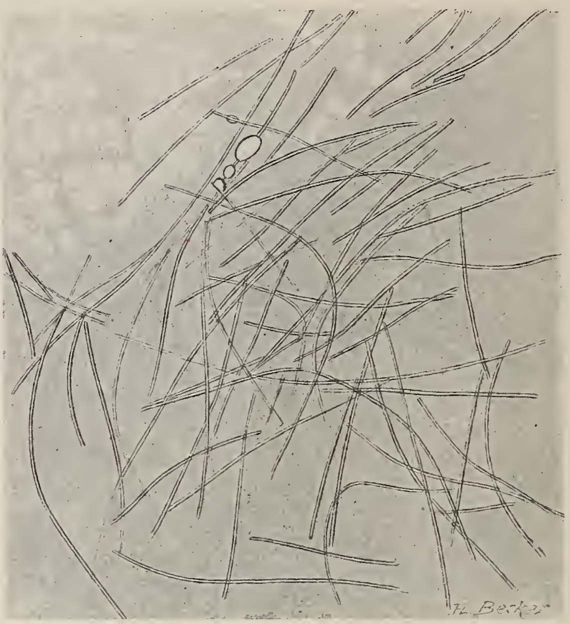


FIG. 6.—Fatty acid crystals resembling elastic tissue in the sputum of a case of bronchiectasis. × 400.

FIG. 7.—A leptothrix form in the sputum, resembling elastic tissue. × 400.

coarser and of more irregular outline (see Fig. 8). Moulds should be easily recognized (see page 37).

The presence of elastic tissue is the surest sign of disintegration of the lung. About 90 per cent. of the cases are of tuberculosis. In this disease it has a certain prognostic value, since as the healing process begins it becomes scarce and then finally disappears. Its constant presence means an advancing disease. In tuberculosis it is present usually in minute particles. In gangrene of the lung it can almost always be found despite the old idea which attributed a certain diagnostic importance to its absence. It was then supposed to be digested by a ferment, but Dr. Osler states that he has never seen a case without it,

Fig. 8.—Elastic tissue from saliva; origin, the food. × 400.

and usually in large fragments. It is also found in cases of lung abscess, of liver abscess perforating through the lung, and finally in fragments of tumors.

Crystals are present only in sputum which has stagnated in the body for a long time or stood in the cup. They occur chiefly therefore in putrid bronchitis, bronchiectasis, tuberculosis, and gangrene. *Fatty acid crystals* occur either singly or in rosettes, short or long. They usually are found in clusters in a mass of detritus. When very long they may simulate elastic tissue (see Fig. 6), but they are usually relatively thick, with stiff curves and pointed ends. If pressure is made on the cover-glass, varicosities will result. They are soluble in potassium hydroxide and in ether. (The specimen should be dried

before the ether is added.) If the slide be warmed, the crystals will disappear and fat droplets take their place. It is necessary to exclude elastic tissue and chains of bacilli. *Cholesterin* crystals are rare; they occur usually in company with fatty acid crystals. *Tyrosin* and *leucin* occur chiefly in putrid sputum which has decomposed in the air-passages. If the sputum be evaporated in the air, the crystals will separate out. In the case of abscess of the lung they are sometimes found in the first discharge, not later. Tyrosin is present as sheaves of long black refractive needles. The spherules of leucin, which perhaps never occurs without tyrosin, are more seldom found unless the sputum be evaporated. It is necessary to exclude soap globules (see page 361). *Triple phosphate* and *calcium oxalate* crystals occur in the same conditions. The rhombs or the needles of *hæmatoidin* occur especially in abscess of the lung, perforating empyema, or a liver abscess perforating through the lung. They are found seldom after hemorrhage, for in this condition the hæmoglobin is changed to amorphous granules. For figures of these crystals, see chapter on Urine.

The *Charcot-Leyden crystals* (see Fig. 67) resemble a diamond very much elongated, or, to use the description of one, two very sharp pyramids with the bases together. They have sharp, elongated points with clear-cut edges, colorless, with very little yellow refractivity. They are brittle, hence easily broken in making the specimen. They vary greatly in size, some requiring the oil immersion lens, while the largest are 0.075 mm. long and 0.04 mm. wide. They stain with eosin, are soluble in hot water, mineral acids, and the alkalies. They occur singly or in groups, in which latter case they may form clusters. In these groups it was noted that on cross-section they were hexagonal, and therefore are not octahedral crystals, as was formerly supposed.[9] Other forms, for instance a Greek cross, may occur but rarely. That they belong to the hexagonal system is very sure proof that they are not the Böttcher spermin crystals. Their appearance had identified them with these, but the similarity is only superficial, and the proof that they belong to a different crystal system rules out this identity, hence there is no reason for supposing they consist of spermin. They are quite certainly derived from eosinophile cells, but how is uncertain. They occur wherever these cells are increased, especially when the sputum has been stagnant, and increase in the sputum after expectoration if it be placed within the thermostat. They occur, therefore, in the largest numbers in the sputum of asthma, in which case 60 per cent. or more of the leucocytes may be eosinophiles. According to a former idea, it was the irritation of these sharp crystals which provoked the asthmatic paroxysms. In this condition they occur chiefly in connection with the Curschmann spirals. They occur, however, in other con-

[9] Cohn, Deut. Arch. f. klin. Med., 1895, liv. 515.

ditions than asthma, and while some claim that in this disease they are not always present, others have found them in every case in which they have searched for them.

Plant Parasites.—The bacteria present in large numbers in the sputum are, with the exception of a few specific germs as of tuberculosis, whooping-cough, influenza, pneumonia, and a few others, chiefly saprophytes which are added in the mouth, or by the cup or air after expectoration, and which increase enormously in the sputum on standing. In other cases these harmless organisms live in the respiratory passages, as in bronchitis, and especially in bronchiectatic and tuberculous cavities. These are simply saprophytes, and if they have any effect it is only to aid in the decomposition of the sputum. These masses of saprophytes sometimes deceive a student, since he suspects some specific germ when he sees a large epithelial cell full of small diplococci, or zooglia of bacilli. The chromogenic bacteria are of importance macroscopically rather than microscopically, since during the warm months they may entirely change the color of the sputum after it has been expectorated. Interesting ward infections, that is infections of the cups which pass from bed to bed, sometimes occur. Streptococci and staphylococci and other truly pathogenic organisms are found in large quantities in certain diseases, and may, in tuberculosis, aid in the destructive process of the lung.

Streptothrix Pseudo-Tuberculosa.[10]—This streptothrix was found in a man with extensive consolidation of both lungs, but who clinically had had no sputum. The symptoms were generally those of pulmonary tuberculosis. Warthin and Olney[11] collected a group of five cases, including their own, with, they think, the same organism (Streptothrix eppingeri) in the sputum, cases with the clinical picture of pulmonary tuberculosis. This organism has true branching threads occurring in large entangled masses, even grossly visible as minute grayish granules in a white, homogeneous, not bloody sputum. Some of the filaments are very long (even several oil-immersion fields), with short branches and without club-shaped ends. They are about four times as thick as the tubercle bacillus. They are acid-fast, staining with a beaded appearance, but are slowly decolorized by 95 per cent. alcohol. They stain by Gram's.

The leptothrix group of normal mouth organisms flourishes in abundance in the lungs, especially in putrid gangrenous disease. Their probable effect is to aid in the decomposition of the sputum. Miller has separated from the old group of "Leptothrix buccalis," *Leptothrix innominata*, an organism unsegmented, straight, but sometimes wavy, from 0.5 to 0.8 micron broad, which occurs always in the tartar of

[10] Flexner, Johns Hopkins Hosp. Bull., June, 1897.
[11] Am. Jour. Med. Sci., 1904, cxxviii.

teeth. This cannot be cultivated, and with iodine solution stains a pale yellow. *Bacillus buccalis maximus* is an organism occurring in single threads or in bunches of parallel threads, the single organisms from 30 to 150 microns long and 1 to 1.3 microns broad, joined into long threads. These cannot be cultivated and take a deep blue with iodine. *Leptothrix maximus buccalis*, a somewhat longer parasite than the last mentioned, but otherwise similar except it does not give the iodine reaction.

The MICROCOCCUS TETRAGENUS is a parasite occurring always in groups of four in a mucous capsule, each organism about 1 micron in diameter. It is a pyogenic parasite which occurs in the sputum in bronchitis, in tuberculous cavities, and hemorrhagic infarctions. It may aid the tubercle bacillus in its destructive processes. To recognize the pathogenic form it must be cultivated, for there is in the mouth of normal persons a harmless parasite of exactly the same appearance, which, however, cannot be cultivated.

SARCINÆ are rare in the sputum, and when they do occur are probably harmless saprophytes. They occur chiefly in gangrene, tuberculosis, bronchitis (see page 68), pneumonia, and in the sputum of old debilitated persons. They are the cause of the gray patches of stomato-pharyngomycosis sarcinica. Whether this parasite is the same as the sarcina ventriculi or not is a disputed point.

YEASTS occur but rarely are recognized. Fresh sputum must be examined. They are usually accidentally present, since, although universal, they flourish only in the fluids suitable for them, and in man these fluids are rare excepting the urine of diabetes and the gastric juice in certain cases of dilatation. They occur often enough, however, and should not be overlooked or unrecognized. They are oval or elliptical cells (see Fig. 64), rarely spherical, so refractive that they may resemble a fat droplet so markedly that chemical reactions are necessary to differentiate the two. Their size varies from 1 to 40 microns in diameter, although of each yeast there is a recognizable average size. Their appearance varies, in some cases being naked cells, in others with a membrane, or a membrane and vacuoles, according to the age of the cell. In some the nucleus is evident even in the fresh cell. The characteristic feature of a yeast is its reproduction by budding; that is, the projection from any part of the cell of a small bud which grows and then constricts off. Although for the most part extraneous, the presence in the sputum of certain pathogenic yeasts cannot be denied, and in doubtful lung cases they should be looked for. Busse[12] has discussed at length his case of "saccharomycosis hominis," an infection by a pathogenic yeast, "Saccharomyces busse," of the tibia, resulting in caseous cavities in both lungs in which the yeast

[12] Kolle and Wassermann, Handb. der path. Mikroorg., p. 669.

was present. The yeast cells were rather small, about 8 microns as an average size although they varied much, very refractive, resembling fat droplets except with a greenish shimmer. The younger cells were homogeneous, but later their membrane and granular protoplasm could be clearly seen. They are made clearer by the addition of sodium hydroxide. It is possible that did we search for such yeasts and not pass them by at once as simple saprophytes, more such infections could be found among our anomalous lung conditions.

MOULDS are commonly enough found, since the air is simply alive with their spores. For their recognition the examination of the fresh specimen is indispensable. Many of them are truly pathogenic, and it is remarkable that there are not more cases described as primary infections. Occurring chiefly in the ear of man, their next seat of predilection is the lung.

These moulds are found only in destructive processes of the lung. Whether these "broncho-pneumonomycoses" are primary or secondary has been a much disputed point with the weight of evidence at present in favor of the primary nature of certain of them. According to the former idea (Virchow) they were only secondary, or formed cavities in the areas of hemorrhagic infarctions. It is a peculiar thing that the cavities containing them are odorless, and there would seem to be an antagonism between moulds and the bacteria of decomposition so that a cavity filled with the former is protected against the latter, and *vice versa*. Granted a destructive process, these moulds would certainly aid and could crowd out the primary organism, hence when examined the case will appear to be one of primary mould infection. Recently, however, through the work of the French, also of Saxer and others, it seems probable that Aspergillus fumigatus can be the primary invader, causing by necrosis an odorless cavity. Among these moulds are:

(1) *Mucor*, of which there are one hundred and thirty varieties, six of which are known to be pathogenic. This is a very common air form. It is characterized by a much-branched unicellular mycelium, which later, however, may have septa; by the form of the sporangia which are at the end of erect hyphæ, and consist of a columella surrounded by the spores, the whole enclosed by a membrane. Fig. 9 represents Mucor mucedo, a very common harmless form. If such a mould be found in the sputum, it is well that the observer note carefully the shape of the columella, the size of the spores, and the nature of the membrane, although for certain recognition cultures are necessary. The varieties known to be pathogenic are Mucor corymbifer, which is a fine delicate small mould with spores 2 by 3 microns in size, the sporangia colorless, pear-shaped, and of a great variety of size from 10 to 70 microns; its membrane transparent.

The columella, evident only when the spores have dropped off, is top-shaped the large end distal, and colorless. This form has been found perhaps most often in man as the cause of kerato-, oto-, pharyngo-, and pneumonomycosis. Mucor rhizopodiformis, of which the sporangia-bearing hyphæ are single or branch as in a sheaf, short and of a brownish color. The sporangia are globular, when ripe of a black color, with an opaque membrane, soluble in water, and columella which is brownish, from 50 to 75 microns wide, constricted at the base, which is also truncated and with a wide flat apophysis, to the margin of which the membrane is attached. The spores are colorless, spherical, and from 5 to 6 microns in diameter. Mucor racemosus, the spores of which are from 5 to 8 microns long and 4 to 5 microns wide and round; the columella elliptical in shape. Mucor

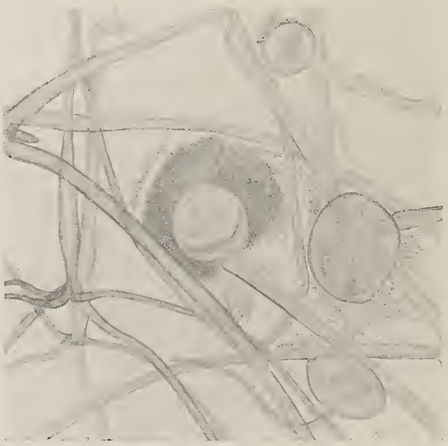


FIG. 9.—Mucor mucedo. × 60.

pusillus, the sporangia of which are black with a thorny membrane, and from 60 to 80 microns wide; the columella egg-shaped or spherical, light brown, from 50 to 60 microns wide; and the spores very small, round, colorless, from 3 to 3.5 microns in diameter. Mucor septatus has a pale, grayish-brown, spherical sporangium, small colorless columellæ which after the loss of the spores may grow still further. The hyphæ have septa, hence the name. The spores are about 2.5 microns in diameter. Mucor ramosus, the sporangia of which are 70 microns in diameter, black in color, with a transparent membrane; the columella round, the spores colorless, opaque, from 3 to 4 microns wide and 5 to 6 microns long.

These forms are known to be pathogenic; almost all of them have been demonstrated in the ear. It is interesting that in all literature only four cases are cited in which they have been demonstrated in the lung,

and so far as we know in none of these cases were they found in the sputum before death.

Aspergillus fumigatus (see Fig. 10). This is by far the most important pathogenic mould. Its mycelium is a thick mesh of threads from 3 to 6 microns wide, the finest without septa but the oldest with. The conidia-bearing hyphæ are short, club-shaped, from 8 to 10 microns in diameter at the larger (distal) end. The sterigmata are unbranched, from 6 to 15 microns long, and packed together from a central point, thus giving a fan-like appearance. The conidia, a chain of which is at the end of each of the sterigmata, are round, colorless, and from 2.5 to 3 microns in diameter. All parts of this mould have a brownish to a dark grayish-green color. The size of the spore is important, since those of Aspergillus glaucus are from 7 to 8

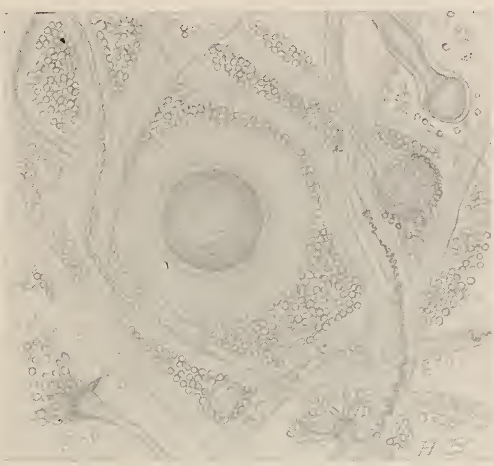


FIG. 10.—Aspergillus fumigatus. × 300.

microns in diameter. The spores occur everywhere, as can be demonstrated by exposing a moist piece of bread to the air for only a few minutes and then placing it in the thermostat. Aspergillus flavus (see Fig. 11), has conidia-bearing hyphæ which are from 7 to 10 microns thick, with the head of a yellowish or green color according to whether it is dry or wet, and brown when old. The conidia themselves are round, of a sulphur-yellow color and from 5 to 7 microns in diameter. Aspergillus niger is of a chocolate brown color, and the conidia from 3.5 to 5 microns in diameter. Aspergillus subfuscus is of an olive-green to a black color, resembles much the fumigatus, but is more pathogenic. Of these forms the Aspergillus fumigatus is the only one that has been shown to bear a direct relation to that pathological process known as "pneumonomycosis aspergillina." Sticker has collected from all literature

twenty cases in which no other disease of the lung was present. Of these, in sixteen was found Aspergillus fumigatus, in four cases the mould was doubtful. One of these, reported by Osler, was a woman who for twelve years had expectorated masses of mycelium the size of a bean, grayish and of a downy consistency; in five cases the mould could not be classified. An interesting case of primary chronic "membranous" bronchitis due to the Aspergillus fumigatus was reported by Devillers and Renon.[13] The patient was a grain-sorter. Fragments of membrane composed of the mycelium of this mould (recognized from cultures) were expectorated monthly. They were from 1 to 6 cm. long, and, having no branches, probably arose in the larger bronchi. In nineteen cases with the Aspergillus fumigatus the infection was mixed. Sticker[14] has divided the cases into the

FIG. 11.—Aspergillus flavus. $\times$ 300.

"sporadic," which are of old feeble subjects or persons suffering from a lung disease, and the "endemic," in which case the disease is due to the occupation of the patient. The two best illustrations of this latter have been described by the French writers. The first consists of a pseudo-tuberculosis present in pigeon-feeders, who are much exposed to the moulds of grain, and hair-combers who work in an atmosphere so laden with infected dust that the cat is the only animal that can live in their neighborhood. No autopsies have been made on such cases. Clinically, the course is often similar to that of a chronic pulmonary tuberculosis. At the onset there is hemorrhage in some cases, either slight or profuse, and which is generally repeated at intervals. The cough is dry at first, then accompanied by a frothy

[13] La Presse Med., 1899, ii. p. 325.
[14] Schimmelpilzkrankh. der Lungen., Nothnagel's System, 1900, xiv.

sputum which quickly becomes greenish in color and purulent. This may continue for months or even years. Blood flecks are often present. Toward the end the hemorrhage may recur or not, the expectoration is of a greenish color, purulent in nature, and nummular in character.

Another form is a chronic bronchitis resulting in final cirrhosis of the lung. In this case the sputum is abundant, foamy, and watery. In Wheaton's case [15] the condition simulated actinomycosis with anatomically the presence of a few tubercles and a large cavity. In some cases there have been casts of the bronchi formed of mycelium and conidia expectorated.

For diagnosis these moulds must be demonstrated in the sputum, and in any case of suspected tuberculosis without the tubercle bacillus

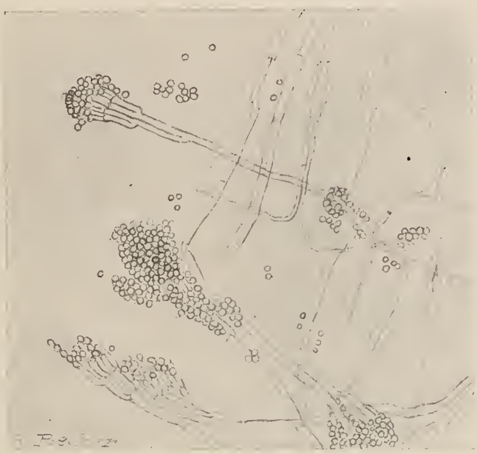


FIG. 12.—Penicillium glaucum. × 300.

they should be searched for. There may be present either the mycelium, the conidia hyphæ, or the spores. As a rule, they are overlooked or passed by as extraneous. The odorless character of the sputum is an interesting fact, even in cases of marked gangrene of the lung with the expectoration of large masses of lung-tissue. In some cases the absence of the mycelial threads may be explained by their destruction, which certainly soon occurs.

The *Penicillium glaucum* (see Fig. 12), which is the most common of our media contaminations, has segmented conidia-bearing hyphæ which divide brush-like at the end, the branches being tipped by sterigmata which are flask-shaped, bearing conidia from 2 to 3 microns in diameter. The refractile conidia of this mould are so common that it is strange that the students do not recognize them oftener, particularly

[15] Trans. Path. Soc., Lond., vol. xliv. p. 34.

as some show the characteristic sprouts. The mould is non-pathogenic. The Penicillium nummula is certainly pathological for animals, and has been found in the ear of man.

Although the general class, mucor, aspergillus, or penicillium may be recognized if the sporangium or the conidia head is found, yet a closer differential diagnosis can only be made on cultures. This may be done by spreading the sputum over a piece of bread as media, or using Sabourand's media (maltose, 3.7; pepton, 0.75; water, 100).

The moulds may be stained in the fresh specimen by a saturated watery solution of saffranin or, better still, of thionin.

Oidium albicans is the very common parasite of thrush, which can develop in the lungs as well as at its common seat, the mouth and pharynx. It occurs chiefly in the mouths of children during their first week, especially in the weak babies; more rarely in older children or adults weakened by old age or disease, especially diabetes or typhoid fever. In these cases we have secondary growths in the throat, nose, œsophagus, bronchi, and lungs. The most common form is the large-spored variety. It may occur in the sputum in two forms,—the first, the yeast-like cells, from 5 to 6 microns long and 4 microns wide, and oval, which in shape cannot be told from any other yeast; and the threads of all sizes and lengths with a double contour containing droplets, granules, vacuoles, but especially conidia-like bodies which are true endogenous spores.

Actinomycosis infection of the human lung is rare. It sets up a chronic process, but one which progresses unrelentingly till death. In some cases there is a slight catarrhal bronchitis for a long time, but the most common form is, from the onset on, a bronchopneumonia. The consolidated areas break down forming cavities which contain fluid, pus, fatty detritus, fat globules, degenerated red blood-cells, and the sulphur granules. Clinically, the picture is of tuberculosis, and yet if the sputum be watched carefully an early diagnosis can be made. Other cases are of chronic bronchitis, while still other cases present the picture of a miliary tuberculosis. The abscess cavities may be very large. The sulphur granules are the characteristic find. They are small granules, in size varying from microscopic to 2 mm. in diameter, of a yellowish, grayish, greenish, or brownish color, round, sometimes abundant in number, in other cases, few. Microscopically they are a net-work of fine twisted threads, straight or wavy; at the ends of many at the periphery are the characteristic club-shaped swellings which when present in large number form a ring around the granule to which they give a radiating or star-like appearance. In general it may be said that in any case of atypical lung disease always think of this (Osler). The expectoration is usually mucopurulent, sometimes fetid. It may be simple mucus, very scanty, or may be purulent and hemor-

rhagic. It is said that the sputum is sometimes as rusty as in pneumonia. It is also possible that the patient may say that he has expectorated at one time a large amount of offensive yellow material. Tubercle bacilli will not be found, neither will elastic tissue.

Animal Parasites.—INFUSORIA are rare and unimportant. There has been described the *Amœba pulmonalis* (Artault), "a small amœboid cell which when dead and stained looks exactly like a leucocyte, but while motile differs from it in its refractility and staining qualities." The true amœba coli may be found in the case of perforated liver abscess and in cases of abscess of the jaw communicating with the mouth (Flexner). It is important that the student should bear in mind that the same rule obtains here as in the examination of the stool, and that nothing should be called an amœba unless its amœboid motion has been clearly seen.

Of the FLAGELLATA, *Trichomonas pulmonalis* is the name given to a form which has been found several times in the sputum. A. Schmidt found them only in the Dittrich's plugs, while Artault found them in the contents of a large tuberculous cavity. These may be the forms which others have found in lung gangrene and putrid bronchitis. In a recent case of large abscess of the lung following pneumonia, and operated on six weeks after the onset of the pneumonia, the sputum contained large numbers of these flagellates. It is probably the same as the Trichomonas vaginalis. *Cercomonads* have been found in the sputum and in the Dittrich's plugs of lung gangrene.

ECHINOCOCCUS DISEASE.—Next to the liver the lung is the most common seat of this infection (in 4.5 per cent. of cases) (see Fig. 55). If the cyst bursts we may find in the sputum the daughter cysts, scolices, hooklets, or fragments of membrane, any one of which is characteristic. They may also be derived from a liver cyst which has perforated through the lung. The cyst wall consists of two layers, —an external laminated cuticular capsule, and an internal granular parenchymatous endocyst. Fragments of this laminated capsule are characteristic of the disease. The cyst content is a clear, limpid fluid, from 1005 to 1015 specific gravity, which contains no coagulable albumen, is neutral or slightly acid, and contains considerable sodium chloride. Inosite, leucin, tyrosin, succinic acid may be found, also hæmatoidin. From the endocyst buds develop which grow into smaller daughter cysts, which then break loose and lie free. Inside these daughter cysts may develop granddaughter cysts. From the inner wall of any of these cysts may develop brood-capsules, cysts from the inner or outer wall of which the scolices develop. A scolex is the head of a Tinea echinococcus, and presents a rostrum with four suckers and a circle of hooklets. If found while alive, the head actively protrudes and retracts this rostrum. In many cases the cyst wall degenerates,

becomes inspissated and filled with a cheesy material which contains masses of free hooklets and dead scolices, which then have a coating of calcium carbonate which effervesces actively on the addition of hydrochloric acid. The presence of these cysts may lead to gangrene of the lung and the formation of cavities connected with the bronchi. Hemorrhages are the rule, usually slight or mere streaking, but sometimes profuse. As a rule, such cases are diagnosed as phthisis or gangrene, unless one of these characteristic bodies is found in the sputum. The cough may be at first dry and hacking, and as the cyst increases in size one with some mucoid expectoration. After the rupture of the cyst and the discharge of its contents the cavity becomes infected, hence that of an abscess, and discharges fetid pus in some cases of a chocolate color resembling the pus said to be characteristic of hepatic abscess. In other cases the suppuration occurs before the rupture and the contents undergo fetid decomposition; then follow the symptoms of rupture of an abscess. If daughter cysts and pieces of membrane are found in the sputum, it means that the bronchus communicating with the cyst is a large one. Pieces of the membrane may be expectorated for months. The rupture is often accompanied by a copious hemorrhage which later may recur.

Paragonimus Westermanii.—This parasite, the "lung fluke," is the cause of the parasitical hæmoptysis of man which occurs so com-

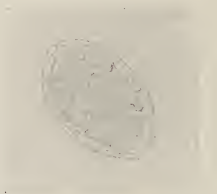


Fig. 13.—Egg of Paragonimus westermanii from the sputum of Dr. McKenzie's case (through the kindness of Dr. Stiles). × 400.

monly in Japan, parts of China, and Korea. Of some mountain towns a majority of the people are said to be infected; in Okayama, 0.4 per cent. of all hospital cases admitted; in Kumamoto, 5.9 per cent. of pulmonary cases.[16] One case in this country which came under the care of Dr. Mackenzie, of Portland, Oregon, has been reported by Stiles,—that of a Japanese who recently immigrated. There was found in domestic animals in this country, previously, a parasite which seems to be identical, and it is perhaps only a matter of time before more and endemic cases will be found among our cases of "tuberculosis." The duration of the disease is long, from ten to twenty years from the appearance of the first symptom. The diagnosis rests with

[16] Inouye, Zeits. f. klin. Med., 1903, vol. l. p. 120.

the discovery of eggs in the fresh sputum. The sputum is generally small in amount, very viscid, consisting of small pellets of blood mixed with mucus, or it may be rusty as in pneumonia. When no blood is present it is of all shades of yellow and brown, but especially a dark, dirty red or brownish color, this color being due to the eggs themselves. Blood is common and yet not constant, often intermittent. Colored spirals resembling grossly the Curschmann spirals are quite characteristic. The eggs are the only characteristic symptom, and may be expectorated in large numbers. The amount of blood while usually small, at the onset only in drops, may be large, from 300 to 800 cc. in a few hours, especially if the patient leads a laborious life. There may be rather large hemorrhages which recur with great frequency, or the disease may take a very slow course. The hemorrhage is always arterial. Its cause is not clear, and it seems more accidental than otherwise, since the ova are not in the pellets of the blood, but in the rusty portions. The eggs have a thick, smooth shell of a dirty reddish-brown color, and a characteristic lid at one end, not seen in some eggs, not exactly on the end of some, and partly shelled off in others. They are 68 to 96 microns long, 48 to 60 microns wide. Charcot-Leyden crystals are common in the sputum, " sufficient proof that such crystals do not explain asthmatic paroxysms, since these cases never have asthma."[17]

Chemical Examination of the Sputum.—The chemical examination of the sputum is seldom of much importance, and those tests which have been proposed have as their object to demonstrate the relative amounts of mucin and albumin. The Zenoni modification of Schmidt's method (who stained the sputum with the Grübler-Biondi stain) is perhaps the most valuable of the muco-chemical. He spreads a small particle of the sputum on the cover-glass, treats it with alcohol for at least a quarter of an hour, and then stains with a half-saturated water solution of saffranin. The specimen then held against a white base shows the mucus as yellow, the albumin as red. It has the further advantage that when many pus-cells are present, and hence the picture obscured, the color of the ground substance can be determined microscopically. The value of such is to demonstrate the difference between pneumonic and other processes, in pneumonia the amount of albumin being considerable.

The chemical test for soluble albumin is simple. The sputum is mixed with 3 per cent. acetic acid, shaken well, allowed to stand twelve hours, and filtered. The filtrate is tested with potassium ferrocyanide, or neutralized, sodium chloride added, and the heat-acid test used. Quantitatively it may be estimated by the Esbach tube. In the filtrate

[17] See Stiles and Hassall, Sixteenth Report of the Bureau of Animal Industry, 1899.

of the heat test the albumoses may be precipitated by zinc sulphate, or determined by the nitrogen present. Deutero-albumoses alone have been found; no peptone (Wanner). Wanner found by far the most soluble albumin in the sputum of pneumonia,—0.3 to 3.6 per cent.; least in that of bronchitis (a mere trace); in that of practically normal persons the merest trace, if any at all. Its presence means inflammation, not hypersecretion alone. Anything more than a faint opalescence is pathological.

Mucin he determined from the glucosamin formed by adding to a weighed amount of sputum two volumes of alcohol, shaking, filtering through a hardened filter paper, and washing with alcohol. The precipitate is then boiled with 10 per cent. HCl for three hours in a flask with return-cooler. The flask is then quickly cooled, made alkaline with NaOH, then acid with acetic acid, then precipitated with phosphotungstic, to remove the biuret-giving bodies, and the reducing substance determined with Fehling's solution (glucosamin having the same reducing power as glucose). Pure mucin contains 33.6 per cent. glucosamin.

Much mucin (1 to 3.3 per cent.) was found in chronic bronchitis, a moderate amount in pneumonia (0.66 to 1.03 per cent.) and phthisis (0.74 to 0.79 per cent.), none in bronchiectasis. Sputum is digested rapidly by autolysis.

As to the value of these chemical tests, which are easy and rapid; Wanner considers that a definite trace of albumin in a case of incipient tuberculosis or chronic bronchitis will mean the former; much albumin indicates pneumonia or pulmonary œdema, and in a case of either pneumonia or infarction of the lung, the former.

Peptone in the sputum has been claimed by some, denied by others, and a trypsin-like ferment is assumed in gangrene of the lung to explain the partial disappearance, perhaps complete in some cases, of elastic tissue.[18]

Pulmonary Tuberculosis.—"Pulmonary tuberculosis has no characteristic form of sputum" (Brown).

Cases of the FIBROID FORM may have no or little expectoration, and this free of tubercle bacilli for a long time. But, as a rule, there is a purulent sputum.

In ACUTE MILIARY TUBERCULOSIS the sputum, if any at all, is that of bronchitis, hence mucopurulent or blood-streaked. No tubercle bacilli need be present. In the ACUTE PNEUMONIC TUBERCULOSIS with extensive caseous consolidation there may for one or two months be no sputum whatever, but, as a rule, it is that of a typical acute lobar pneumonia, rusty until the crisis should come, and then when one expects

[18] See Wanner, Deutsch. Arch. f. klin. Med., 1902, lxxv. 347; Fr. Müller, Ztschr. f. Biol., Bd. 52. In this is the best discussion of the mucin and allied bodies of the sputum.

it to change to a mucopurulent a green color may be the first indication that there has been a mistake in diagnosis. In the case, therefore, of lobar pneumonia with delayed resolution, especially if the sputum be green, search should always be made for tubercle bacilli, for soon they and elastic tissue may be present, sometimes very early.

Among our cases of acute tuberculous lobar pneumonia (fifteen in number), in four the sputum was typically rusty. In the majority it was a mixture of a rusty with a bronchitic; in two there was almost no sputum at all. The green color and tenacious quality were marked in a few; in two cases the sputum was of a white, sticky, mucopurulent nature from the very first; one marked feature in nearly all cases was the constant blood-streaking, and in two, brisk hemorrhages. In the typically rusty sputum very few pus-cells were present, but many alveolar epithelial cells and red corpuscles. Later, that is after the first week, the sputum is rather mucopurulent, and yet in many continues blood-streaked. The greenish color was marked in several. Later it may become nummular, and in two cases positively foul.

In case the patient had a slight bronchitic sputum preceding the onset of the tuberculous pneumonia the sputum at once changes its nature, becoming tenacious, slightly less in amount, and blood-streaked. If it be a true acute lobar pneumonia which occurs in the course of a chronic tuberculosis, the sputum is that of lobar pneumonia with an admixture of the bronchitic.

Among our cases the following points were particularly interesting. In one the mixture of the rusty and bronchitic sputum expressed itself by the two layers in the cup, the upper mucopurulent and blood-tinged, the lower exceedingly tenacious and stringy. Later on it became greenish, purulent, and very tenacious, then of a tenacious greenish-gray color, which continued until death a short time later. Another case is interesting, since the elastic tissue was found before the tubercle bacilli, although repeated examinations for the latter were made. In one case diagnosed at first as acute lobar pneumonia, on the third day the sputum was markedly blood-tinged, tubercle bacilli were present, and two bronchial casts about 1 mm. in diameter at their larger end. In a case with sudden onset two days before admission and without previous history the sputum was white, sticky, and mucopurulent. This continued with a greenish tinge for nine days, but on the nineteenth day there was a sudden marked change, the sputum now abundant forming easily two layers, the upper sanguineous, the lower mucopurulent, blood-tinged for the first time, and on that day, the nineteenth, the tubercle bacilli were first found, although they had been repeatedly searched for.

In the ACUTE TUBERCULOUS BRONCHOPNEUMONIA a hemorrhage is sometimes the first symptom. The sputum may early show elastic tissue and tubercle bacilli.

In the CHRONIC ULCERATIVE TUBERCULOSIS, the sputum may assume almost any color or any form assumed in any other disease. Biermer divided it into four forms; the mucoid, mucopurulent, blood-stained, and pure blood. It may vary in amount from none to one litre in twenty-four hours; in consistency from that of extreme tenacity to very watery. In some cases, especially those of the early

apex tuberculosis in which the physical signs are marked and a cough present, there is no sputum. For weeks there is usually a slight morning sputum, but it may be necessary to urge the patient to expectorate this; in other cases the onset is with a slight hemorrhage; in other, it is at first a pure mucus and hence glairy, containing much myelin, giving it the sago appearance. There is nothing distinctive in the gross appearance of this sputum. It may last for months, but sooner or later there will appear little caseous lumps, one of the first suggestive signs of tuberculosis. Later the sputum is more profuse and mucopurulent, that of chronic bronchitis. As ulceration proceeds it becomes more profuse, yellow or greenish in color, and after that of any grade or amount, and from mucopurulent to purulent, or even pure pus. Microscopically, it contains epithelial cells of all kinds, pus-cells, blood, and in some cases, if the sputum be hardened *en masse* and sections cut, giant-cells may be found.

The *variations* in the sputum in chronic ulcerative tuberculosis are many. "A sudden disappearance of the sputum when before it had been abundant, especially in the morning, should always put us on our guard. Miliary tuberculosis is occasionally ushered in in this manner" (Brown). In cases of sudden heart failure there may be a cessation of sputum for one or two days without apparent injury. An abundant mucoid more or less frothy sputum marks the onset of miliary tuberculosis in a chronic or a less acute form (Brown).

One of the most important points in the sputum examination is the recognition of the small caseous particles, "rice bodies" (corpora oryzoidea). These should never be searched for at random. The sputum should be spread out on a dark plate, or better still, according to Sir Andrew Clark's method, squeezed between the surfaces of two plates of glass, and then the whole surface scrutinized with a small hand-lens. This is the surest way to find these particles, in which one has the best chance of finding tubercle bacilli and elastic tissue. Many prefer wide Petri's dishes, which are more easily handled and sterilized. These small caseous particles are from about 0.5 to 1 mm. in diameter, of a white opaque color, more or less rounded in shape, of a bad odor, and when picked up with a needle and spread on a slide are found to be more brittle and crumbly in character than particles of bread. Casts of tonsillar crypts and glands of the trachea, bronchi, and pharynx are to be excluded. Several of these are collected on a slide and squeezed out under a cover-glass in case elastic tissue is looked for, but if for bacilli they are spread by means of another slide, or, what is better, spread with a needle the slide held a little distance above a gas flame, and stained.

Elastic Tissue (see page 29).—The search for this should never be at random. It should always be methodical and intelligent, for one

can search long and find none, whereas, did he know what particles to choose, he might find plenty in the first specimen. Its presence means destruction of the lung. As a rule, in tuberculosis the disintegration is molecular and the elastic tissue in very fine particles, grayish threads, even in single fibres. This tissue may present the arrangement of the alveoli, or come from the bronchi or from the blood-vessels. Brown spoke of a case in which a little pouch of elastic tissue 8 to 10 mm. in diameter, evidently the wall of a cavity, and containing innumerable tubercle bacilli, was expectorated. In certain cases it will be found early before there is any suspicion of disintegration; in other cases death may ensue before any is found, as, for instance, in caseous pneumonia.

Tubercle Bacilli (see Fig. 14).—The search for these, the most important proof of tuberculosis, should be made with especial care. The caseous particles already mentioned and described should always be selected if present; if not found, small bloody or purulent masses. The bacilli may be present in the bloody masses of the initial hæmoptysis. One must not give up in case no promising particles are found, for bacilli may occur in good numbers in the watery mucoid sputum as well as in the mucopurulent. It is better to exhaust the resources of a careful search by the Sir Andrew Clark method for suitable particles before the more elaborate methods are employed. Five or six portions of the sputum should be examined. Since it is often desirable to demonstrate the few bacilli sometimes present, many methods have been proposed. These nearly all rest on the fact that the sputum may be rendered homogeneous, then the organisms sedimented to the bottom or salted to the top of the fluid.

To render the sputum homogeneous it may be boiled with an equal amount of 5 per cent. KOH; or diluted with two volumes of water and then KOH added until 2 per cent., then, if necessary, heated; or what is better, saturated calcium hydroxide, 10 parts, is added; or 5 per cent. carbolic acid nine parts to one of sputum; or the sputum is digested, 5 to 10 ccs. of sputum being mixed with ten volumes of 0.2 per cent. soda to which is then added 0.5 gm. pancreatin and it put in the thermostat for from six to twenty-four hours. A little phenolphthalein is added to make sure the reaction remains alkaline. Of these methods, the heating with KOH is the least useful, since it certainly changes the staining properties of the bacilli.

The sputum now homogeneous, it remains to concentrate the bacilli. This cannot be done by simple centrifugalization, since the specific gravity of the bacilli is the same or lighter than that of the fluid, and after standing even eight times as many may be found in the supernatant fluid as in the sediment. The specific gravity of the fluid must be changed, made lower than 1010 or higher than 1080 (the

limits of the specific gravity of a culture of these bacilli). The addition of an equal amount of alcohol will accomplish the former, but alcohol seems to affect the bacilli; or an equal volume of 25 per cent. NaCl solution added and the bacilli removed later from the upper layers. The specific gravity of sputum varies from 0.929 to 1.2242. It is well to add a little egg albumin to fix the organisms to the glass. Some prefer to make the specimens on cover-glasses; others use slides, which is easier and allows a larger surface. The smears are best made with a needle, holding the slide above a flame at such a distance that the specimen is merely warmed. The mucus then is easily spread.

Staining.—The tubercle bacillus is acid-fast, but as there are other organisms with this property to the same or a less degree, one must be on his guard. The Ziehl-Neelsen carbol fuchsin is generally used (fuchsin, 1 gm.; absolute alcohol, 10 cc.; 5 per cent. carbolic acid 100 cc.). The specimen may be stained either by heat or in the cold. If by heat, the slide is covered by the stain and then held over a small flame of a Bunsen burner until the boiling point is reached. As the stain evaporates, more must be added, as the specimen must be well covered or the glass will crack or portions dry down which it is very hard to decolorize. The stain should boil well for from one to four minutes, or until the crystals of fuchsin are seen on the surface. To boil well for a quarter to half a minute is usually enough; mere steaming is insufficient. The stain is poured off, the smear washed in water, and blotted. By the cold method the specimen is immersed completely for twenty-four hours. If cover-glasses are used they are floated in a watch-glass full of the boiling stain. It should be borne in mind that it is essential to overstain as much as possible, since with even the best technique it is quite certain that only a fraction of the bacilli will hold the stain. The methods of decolorizing vary considerably. One of the best decolorizing agents is 2 per cent. HCl in 80 per cent. alcohol; the smear is decolorized until only the thickest portions are red, then washed in water. Others use 25 per cent. nitric acid until the fuchsin tint is gone. This may be controlled under the microscope. It is then washed in water. If some color returns, this specimen is covered again with nitric acid and again washed in water. It is then washed with alcohol and then with water. This whole process is to be repeated if necessary. When well decolorized, only the thickest parts of the smear will show any red. For counter-staining, Loeffler's methylene blue may be used (the smear covered with this for about five seconds, then well washed). By either of the above methods most of all the other "acid fast" bacilli are decolorized. If, however, there is no doubt about the diagnosis, and one wishes merely to follow the number of bacilli present, Gabbett's methylene blue is used because of its simplicity (1 to 2 gms. of methylene blue, *i.e.*, to saturation, in 100

cc. of 25 per cent. H_2SO_4). The specimen, dried with filter paper, is covered with this for from one to five minutes. It is then washed off and examined grossly. If any pink can be seen grossly the process is repeated. By means of this stain we both decolorize and counterstain at the same time, hence cannot tell when decolorization is complete. The thinner the smear the more quickly is it decolorized. Sulphuric acid certainly "burns" the specimen, and the morphology is never as nice as with nitric acid, since the bacilli seem thicker and the beading is less distinct. In fixing, too high heat should be avoided, since it injures their staining quality; in any doubtful cases it is said to be better to use the cold method. Pappenheim [19] has recommended a stain supposed to be very superior for its differentiating qualities. This seems unnecessary, however, to those who have most experience in the practical side of the work.

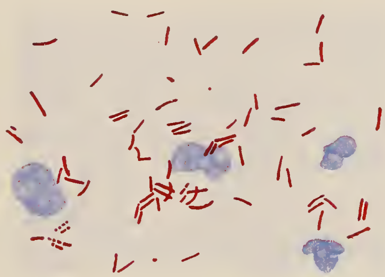


FIG. 14.—Tubercle bacilli, stained with carbol fuchsin, and decolorized with nitric acid. × 900.

Morphology of the Tubercle Bacillus.—As a rule, they are from 1.5 to 3.5 microns long and 0.2 micron wide. Sometimes, however, they are very much longer, even 11 microns. As a rule, they are bent, sometimes much curved, and branching forms have been described. They may occur in chains. The curving may be so extreme that they present a spirochæte form. They are often beaded, from one to eight beads in a rod, and resemble a streptococcus. This appearance is now described as vacuolization, that is degeneration. It occurs in all classes of cases. The bacilli are scattered or in clumps, sometimes parallel, sometimes crossed. It is doubtful that the young bacilli stain less intensely than the older.

The acid fast organisms have now attracted much attention, and their list is rapidly growing. Borrel [20] gives, among others in a long list, the Bacillus tuberculosis (Koch); Bacillus lepræ; the smegma group, from which several have been isolated, and which occurs over the whole surface of the body wherever skin secretion collects, in cerumen and other secretions,—also the nose and eye; the milk and butter

[19] Berl. klin. Wochenschr., 1898, No. 47.
[20] Bull. Inst. Pasteur, May 30, 1904.

bacilli (Rabinowitch); those of certain grasses (timothy hay bacillus and the grass bacillus of Moeller); of sewer water; of soil, and a bacillus of manure (Moeller). In man such organisms have been found in urogenital diseases, typhoid stools, lung troubles (pseudo tuberculosis), and pulmonary gangrene. Similar ones are found in tuberculosis of birds, fish, reptiles, rats, and, of course, of cattle which organism is now quite generally granted to be Koch's bacillus. Borrel states that many of these cannot be distinguished from their appearance alone.

But in addition to the acid-resisting property, that to alcohol is also important, hence our stains now include both decolorizing agents. This means that Gabbett's methylene blue is no longer used, with the result that the above list is much reduced so far as danger of mistake is concerned.

Some [21] consider that several of these organisms belong in one group, including the tubercle bacillus, and are closely allied to the actinomycoses. Several organisms from cases resembling tuberculosis have now been described which resemble much the tubercle bacillus. Ophüles,[22] for instance, in five cases of gangrene found acid-resisting organisms in each, "long, slender, more or less curved rods or threads, with irregularly staining protoplasm, which frequently occur in clusters. In all cases it was possible to find individuals which showed true branching." But these all decolorized in acid alcohol, nor were they very resistant to acid.

The opinion expressed by those who have examined several of the above forms is that they decolorize rather easily in acid, and that if one use both acid and alcohol there is little danger of mistake from those organisms which morphologically resemble the tubercle bacillus. Yet it is to be emphasized that these pseudotuberculosis organisms are studied chiefly from cultures, and hence may not, and in some cases do not, have exactly the same staining properties as when in the body secretions. It is not so easy to inoculate an animal and recover these germs as in the case of the tubercle bacillus. The question in the sputum is not so difficult as in the urine, yet it may not be true that every acid-alcohol-fast bacillus is the tubercle bacillus, and animal inoculation is the ultimate court of appeal.

In searching for tubercle bacilli, if the sputum be homogeneous it is well to study many specimens. Ten are recommended by some. In the technique of this clinic, using only selected particles, we consider it is necessary to examine but three. If we find none, we prefer to give a negative result and ask for another specimen, rather than search longer in that same sputum. Bacilli are most numerous in the muco-

[21] See Abbott and Gildersleeve, Centralbl. f. Bakt., 1902, xxxi. p. 547.
[22] Journ. of Med. Research, 1902, iii. p. 242.

purulent or pure purulent sputum of cavities. They are very rare in the fibroid form of the disease, also in the caseous pneumonia before disintegration of the lung. One negative examination is valueless. Some will search for three days, others say six or seven, while in our cases we believe in searching as long as there is sputum, and in one case the bacilli were found only on the nineteenth examination, and Brown tells of one positive only on the twenty-sixth daily trial. All other methods failing, the inoculation into the guinea-pig may be resorted to. "It is of doubtful value to put the sputum in the thermostat that the bacilli may grow."

The *prognostic value* of sputum examination.[23] When it is remembered that possibly many of the tubercle bacilli are not stained at all; that old foci may give off very few and young foci no bacilli at all, however actively they may be forming; that by the occlusion of the bronchus the contents of the focus may be shut off entirely for a time and when expelled the sputum contain a vast number of tubercle bacilli; that they may be present one day, then not again for months; that in the same specimen the organisms may be abundant in one part of the specimen and none in others; that some persons with fatal tuberculosis have no bacilli in the sputum, as, for instance, caseous pneumonia and acute miliary tuberculosis, while in other cases the bacilli are present even before the physical signs; lastly, that in the severe cases with bronchitis the secretion of the bronchi will dilute the sputum and give the appearance of a diminution in the bacilli, it will be seen that one must be very guarded in his use of the examination in forming an opinion of the prognosis. In general it may be said that while the examination of one specimen is of no value for this purpose, repeated examinations are of use. Brown recommends the application of a modified Gaffky's table in following a case (1/12 oil objective, II ocular used).

I. Only 1 to 4 bacilli in whole preparation.
II. Only 1 on an average in many fields.
III. Only 1 on an average in each field.
IV. 2 to 3 on an average in each field.
V. 4 to 6 on an average in each field.
VI. 7 to 12 on an average in each field.
VII. 13 to 25 on an average in each field.
VIII. About 50 on an average in each field.
IX. About 100 on an average in each field.

Cases are thus classified and designated by the Roman numeral.

Among some of the general points it may be said that while no number, form, arrangement, or staining qualities of the organisms is of

[23] Brown, Montreal Med. Journ., October, 1901; Journ. Amer. Med. Assoc., February 21, 1903. The reader is referred to these articles, from which the most of the following paragraph is quoted.

absolute importance, the continued expectoration of large numbers would indicate a cavity; the sudden increase in their number, diffusely spread, very numerous, and an increase in the cellular elements, would indicate disintegration; the steady decrease lasting for some time would, if the physical signs also improve, indicate improvement; the case should be called "healed" only when the bacilli are absent for a long time. On the other hand, the continued presence of large numbers does not of necessity indicate an active process, as, for instance, Fowler's case, in which for fourteen years bacilli in large numbers were constantly present, and yet the case was in fair health and even improving. Such a case, it is needless to say, is the source of the greatest danger to his neighbors, expectorating as he may from three to four billion bacilli each day. Trudeau has mentioned a similar case extending over a period of ten years.

Many have considered that the form of the bacillus is a more important sign than the numbers, the predominance of short rods indicating a rapid growth, while that of long a slower; yet both forms coexist. Brown considers that while in general morphology gives little or no aid, yet a predominance of short rods does indicate a more active process. Others claim that the arrangement is the important thing, that their presence in clumps and parallel groups indicates a lively growth, and groups of short bacilli a bad prognosis. Yet these clumps may be found in all cases, but more often in the severer. Bacilli which stain deeply are considered to possess an especially bad virulence. Yet the exceptions to all such rules are so numerous that they may be held only in a very broad way.

The question is often asked, Is the discovery of a single bacillus of importance? Attention should be called to the fact that while these so-called single bacilli are very often not bacilli at all, in some cases one single bacillus will be found concerning which there is little doubt; that these may have been deposited from the air; some other contaminating means must always be borne in mind, and the discovery confirmed on following days. Yet with careful technique the presence of one bacillus certainly is important. On the other hand, the repeated negative examinations do not necessarily exclude tuberculosis, as in one of our cases, it was only on the nineteenth day of repeated examination that the bacilli were discovered.

A fairly accurate estimation of the number of bacilli may be made,[24] but for clinical purposes is not worth the considerable trouble it takes.

Sputum from a Cavity.—Some of the older writers (Winkel) have considered that the odor of the breath was of particular importance. The stagnant sputum from cavities has a heavy penetrating sweetish odor, and this gives its odor to the breath. In some cases the

[24] Nuttall, Johns Hopkins Hosp. Bull., May, 1891.

sputum from such a cavity in the cup will be odorless, while the breath is most offensive (see page 22).

During cavity excavation the sputum is mucopurulent, expelled in masses which flatten in the cup to form coin-shaped clumps, the so-called "nummulæ." These are seen especially in the dark green or grass-green sputa of caseous pneumonia with cavity formation. They are green or dirty grayish-green in color, isolated, do not coalesce, and consist chiefly of pus; they sink at once in water; their odor is not bad. Some are full of small points, even millet-seed in size, containing much black pigment and elastic tissue, granular detritus, and few pus-cells. The cavity is full of such material. These are not, as was formerly supposed, characteristic of cavity, for masses macroscopically similar arise also in the larger bronchi. When softening is rapid the expectoration of 100 to 150 cc. a day of sputum is not rare. From large cavities most is expectorated in the morning. Blood is often present, which, if retained in the cavity, is expectorated in blackish clots. In case the cavity communicates with the bronchus by a fine hole, there may result the same skein of pus described under abscess of the lung. The sputum often has a sickening sweetish odor. In case bronchiectasis, gangrene, or putrid bronchitis occurs, with decomposition the odor may be foul, but it is remarkable how seldom these occur.

As the cavity clears and becomes lined with connective tissue, the character of the sputum changes considerably. We then have the "sputa globulosa," consisting of balls of a grayish-white color, thick, rounded, shaggy masses,—a conglomerate of mucus, detritus, and pus,—some of which sink in water, but not all. Large tissue fragments are rare unless the connective-tissue proliferation be rapid and dissects off particles of the necrotic cavity wall.

Hemorrhage.—This occurs in the majority of cases, in amounts varying from small flecks to cupfuls. In some cases the number of hemorrhages is so great they are termed "hæmoptysical" cases. Hemorrhage occurring early in the disease is very frequent, but seldom great, and recurs often. This is the so-called "inflammatory hemorrhage," seen especially at the onset of a caseous pneumonia or during acute exacerbations of the consolidation. It has the same significance as in acute lobar pneumonia, the blood escaping by diapedesis or from erosions of the mucosa. Later in the disease, however, the hemorrhages are of a very different character, since then profuse and sometimes fatal, occurring without warning in a person apparently recovering. Such arise from the rupture of the small miliary aneurisms in arteries which cross a cavity or are exposed in its wall.

Croupous Pneumonia.—In true lobar pneumonia very rarely there is no sputum at all, except in the case of very old or very young

patients. At the onset a hemorrhage is sometimes the initial symptom. In other cases the sputum is mucoid and abundant for even four or five days, but very soon becomes bloody, at first from the presence of unchanged red blood-cells. This sequence, mucoid then blood sputum, marks the progress of the inflammation from bronchi to alveoli. The sputum at this stage is remarkably transparent, since the cells are not present in rouleaux but are scattered singly throughout the mass. Soon, however, the sputum becomes rusty, is then characteristic in appearance, and when typical a diagnosis may be made from it alone, even when other signs fail. This rusty sputum is homogeneous, glairy, almost transparent, so tenacious and jelly-like that the cup can often be inverted without the loss of any. The color is due to the transformed hæmoglobin, and microscopically very few red blood-cells can be found. The above-described sputum is present only in cases in which there is not much catarrh of the larger bronchi, which furnishes mucopurulent masses. In amount it varies from about 150 to 300 cc. per day. When small in amount it dries rapidly in the cup, since there is so much albumin, so little mucus.

Blood is a quite constant feature of pneumonic sputum. For the most part uniformly distributed, it often is also present in streaks of varying size, while in other cases the sputum is almost pure blood. If the process extends to another part of the lung a rusty sputum may again become bloody.

In color it is typically of a rusty yellowish-brown hue, but in other cases with physical characteristics the same it is of an orange-yellow, a lemon-yellow, or a grass-green color; in fact, all the possible shades which are seen in subcutaneous bruises. These colors are due to different oxidation stages of unknown hæmoglobin derivatives (Traube). The sputum may appear jaundiced, but this term should never be used unless the skin is icteroid.

Microscopically is seen a transparent background with some red blood-cells which are swollen and pale as a rule, others well preserved; many epithelial cells, columnar or pavement; lymphocytes, granular cells, and oil globules. Chemically, this sputum is characterized by the absence of the alkaline phosphates, the excess of potassium over sodium, an increased amount of sulphates, and a large amount of soluble proteid. The fixed salts, usually about 18 per cent., are in these cases about 26 per cent.

At the crisis the sputum loses its rusty color and becomes mucopurulent, more or less abundant, and finally white mucus. "In no other (disease) is the cycle of sputa changes so marked or of so great diagnostic value as in this disease" (Mackenzie).

In addition to the study of the individual cases, a series of ninety-four were compiled to get some general idea of the relative frequency of the different forms

of sputum in our cases. Twenty-one per cent. of the cases denied to have had any sputum at the onset of the disease; 46 per cent. denied that it was bloody, whereas 33 per cent. stated that the first sputum that was noticed was slightly bloody. During the course of the disease 16 per cent. of the cases had little or almost no sputum. One case was in the hospital seventeen days without any expectoration, and other cases about seven days. In 32 per cent. the sputum was typically rusty; in 39 per cent., not only rusty but blood-streaked; in 3 per cent., very bloody; while in 10 per cent. at no time during the disease was any blood noted.

Variations.—If bronchial catarrh be also present, that is, when a pneumonia supervenes on a chronic bronchitis (Traube), the sputum consists of mixed rusty pneumonic and mucopurulent bronchitic sputa. It is therefore quite fluid. It may not be rusty at all; it may be bloody mucoid pus. In some cases, instead of rusty it is very bloody; *e.g.*, the so-called "hæmorrhagic pneumonia" of the aged. In chronic passive congestion due to heart, lung, or renal disease, we have the characteristic "brick-red" sputum, thin like that of œdema, and very bloody. This is the sputum of "congestion" or "serous pneumonia" (Traube). It is seen when the inflammation proceeds by starts.

The *green sputa* are of particular importance: in cases of delayed crisis and lysis but in which perfect resolution may follow; in a case clinically becoming serious it is an important warning; it may be the first symptom of abscess of the lung, and should always arouse suspicion in a case with an abnormal course; in cases in which the skin is jaundiced it has no significance; and lastly and most important, it may be the first indication that the diagnosis of croupous pneumonia was incorrect, and a search should at once be begun for tubercle bacilli.

The sputum in the case of pneumonia which ends in necrosis or gangrene presents characteristic changes. It soon loses its tenacious and rusty character, becomes more fluid, its color that of coffee, then prune juice, and later chocolate-brown. The red blood-cells disappear. The odor, at first absent, is stale and later decidedly fetid. Granular detritus appears, and then necrotic fragments. Or it may throughout be prune juice in nature. This is rare. From these sputum changes the diagnosis can be made before the tissue fragments appear. The reason for the color has been somewhat questioned since the red blood-cells are described in cases as well preserved.

The *prune-juice sputum* is also of particular importance. It usually indicates a severe type of the disease; in other cases œdema of the lungs superseding on a pneumonia, which occurs particularly in old patients; in other cases it indicates a low type of the disease; while still again, and in these cases without any serious significance, it merely signifies a beginning resolution.

Fibrin coagula are commonly found in a rusty sputum, as often, says Dr. Osler, as the search is made for them. Suspicious masses should be shaken out in water. These may be beautiful branching fibrin casts of varying size, the larger with hollow branches. In some very pretty casts there are found clots and small collections of blood in the lumen at each bifurcation of the branches. In one case the cast

FIG. 15.—Fibrin cast from a case of double pneumonia. Natural size. The patient was a man 65 years of age. The cast was expectorated on the sixth day of a double pneumonia, followed by hemorrhage. Death on the seventh day.

seemed to be from two entire lobes, one of each lung, and hence to cross the bifurcation of the trachea. This cast is pictured in natural size as Fig. 15. *Curschmann spirals,* and, in fact, every constituent of asthma, may be found.

This was beautifully illustrated in Vierordt's case [25] of typical pneumonia but with intense general bronchitis and bloody sputum on the fourth and fifth days, with many fibrin coagula, which were particularly beautiful on the seventh day, at

[25] Berl. klin. Wochenschr., July 16, 1883.

which time also spirals were found and resolution began. From this time on were found beautiful Curschmann spirals; no Charcot-Leyden crystals.

The *Diplococcus lanceolatus* is present in large numbers in the sputum, and may be stained by Gram's, while the capsule may also be demonstrated by the proper methods. The mere demonstration, however, of this organism has little diagnostic importance, since it occurs in other conditions.

In the **subacute indurative pneumonia** the sputum may contain blood, but is seldom rusty. It is usually abundant. There is a decided tendency for it later to become fetid.

Chronic Interstitial Pneumonia.—In this disease the cough is often paroxysmal, and in general the expectoration is copious, of a mucopurulent or a seropurulent nature, and sometimes fetid. Hemorrhage is present in about one-half of the cases.

Bronchopneumonia.—This term, which includes also the hypostatic, and the pneumonia of aspiration, is accompanied by an expectoration which combines the bronchitic with the pneumonic, that is, a mixture of rusty with mucopurulent sputum. Sometimes the transition from a bronchitis to a bronchopneumonia may be suspected from the changes in the sputum; it becomes less in amount, viscid, difficult to expel, and may be streaked with blood, but it is almost never typically rusty.

Influenza.—The sputum in the pulmonary type of this disease as it occurs in epidemics is at first tenacious and scanty, but later increases, becoming often large in amount, a mucopurulent, very thin fluid, in which swim purulent masses. It is often quite bloody. Pfeiffer considers that the most characteristic sputum is greenish-yellow in color with lumps of pus in coin-shaped masses. In this sputum the influenza bacillus may early be isolated in almost pure culture.

But pulmonary infection by the influenza organism is not confined to times of epidemics, but has proven a very common disease. Lord,[26] in 100 non-tuberculous cases with cough, found this bacillus in 60, and of these in practically pure culture in 29. Such cases are mistaken for chronic bronchitis, asthma, or even tuberculosis, there being nothing distinctive in the clinical picture; the organism must be found. The duration in such cases may be months or years, even in one of Lord's cases forty-four years. Since making a routine examination for this organism in this clinic a surprisingly large number of cases has been found. For Lord's method of cultivating the organism the reader is referred to his paper.

To recognize these bacilli in the sputum it is not so much a matter of stain as familiarity with their morphology. They are first decolor-

[26] Boston Med. and Surg. Jour., December 11, 1902.

ized with Gram's, and a good counter-stain then used. Bismarck brown is the best counter-stain, or safranin. (Twenty cc. of saturated alcoholic solution of Bismarck brown diluted with 80 cc. of water.) Methylene blue gives an uncertain color, and it is often hard to say whether the organism has decolorized or not.

Smith's stain is preferred by some, and in the hands of one accustomed to it gives beautiful pictures,[27] but others find it too complicated and unsatisfactory. Very fresh sputum is examined; small tenacious purulent particles are to be selected for examination; very thin smears are made.

A very thin cover-glass smear of sputum is fixed by heat (passing it two to three times through the flame), and covered with aniline oil gentian violet. It is held well above the flame, allowed to steam, but avoiding burning. The excess of stain is washed off with a solution of iodine in potassium iodide (iodine, 1 part; KI, 2 parts; water, 300 parts). Then the specimen is covered with this IKI solution and the steaming continued. It is then decolorized as much as possible

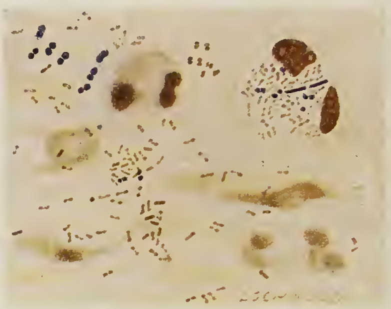


FIG. 16.—Sputum of influenza stained with Gram's and Bismarck brown, showing the influenza bacillus (brown), the diplococcus lanceolatus et al. blue. × 900.

with 95 per cent. alcohol, and washed a few seconds in an alcohol-ether mixture (95 per cent. alcohol, 4 parts; ether, 6 parts). Wash in water. It is then stained a few seconds in a saturated aqueous eosin solution, the excess of eosin washed off with Löffler's blue, then the specimen covered with this blue, steaming as before. Decolorize slightly with 95 per cent. alcohol; wash in absolute alcohol, then xylol, and mount in Canada balsam.

The method used here is as follows: the fixed smear is stained with the above-mentioned aniline oil gentian violet (Sterling's) for one and one-half minutes, and washed in water; covered with Gram's solution one and one-half minutes, and again washed in water; 95 per cent. alcohol, 5 minutes; wash in water; 0.2 per cent. aqueous Bismarck brown, one minute; wash, dry, mount.

The organism (see Fig. 16) is one of the smallest of the bacilli, a short rod with rounded ends, often with polar staining, and which takes at best a rather faint stain. They occur in groups sometimes

[27] Boston Med. and Surg. Jour., December 11, 1902.

of large size and free, and in groups inside leucocytes. The question has been asked if this intracellular occurrence does not indicate a process near its close.

Whooping-Cough.—During the catarrhal stage the cough is, as a rule, that of a dry bronchitis. A little later the sputum of bronchitis presents no especial features, but during the paroxysmal stage the sputum is expectorated by very severe paroxysms of coughing in amounts very small each time, and yet in the aggregate considerable. Such sputum contains almost pure cultures of bacilli.

Glanders of the Lung.—In case the disease extends from the nose to the bronchi and there excites inflammation, the severe cough is said to be accompanied by a profuse purulent expectoration.

Asthma.—In acute bronchial asthma the sputum is perfectly characteristic, beginning, as a rule, only as the paroxysm begins to pass off, or, as the patient describes it, " breaks," and bringing with it much relief. During the paroxysm itself there is often no sputum; in other cases it is scanty, clear, consisting of thick glairy mucous balls, the so-called " perles of Laennec," which swim in a thin clear frothy mucus. In other cases it is less characteristic, of a greenish-yellow tenacious mucus, and described by the patient as " rubber-like." These perles are pellets of a semi-transparent mucus, of a pale gray color like boiled tapioca. In them are mucous moulds of the smaller tubes, and some on unravelling are Curschmann's spirals. Early the sputum contains a few eosinophilic cells and many alveolar epithelial cells with myelin degeneration. The moulds are small cylindrical or sausage-shaped masses consisting of thick threads, or plugs, which may be from 1 to 1.5 cm. long. Some branch, some are narrow or straight, while others are spiral. These have the same significance as the Curschmann spirals. The amount of sputum at this stage may be from very little to 50 cc. In the sputum also may be found alveolar cells with myelin degeneration and very few leucocytes.

In 27 per cent. of our cases there were in some of the paroxysms slight hemorrhages. As the attack "breaks," however, the sputum becomes thinner, more liquid, frothy, and much more abundant, even 200 cc. in twenty-four hours. It is then a clear viscid fluid, which has lost considerable of its tenacity, in which float mucopurulent masses. In others of our cases, however, it was still scanty, tenacious, viscid, yellowish-white, and decreased in amount until it disappeared entirely. The leucocytes are present in large numbers, and a large percentage of them may be eosinophile cells. There are also present large numbers of alveolar epithelial cells, many of them with marked myelin degeneration; few red blood-cells. In one of our cases was present a true bronchial cast about one and three-quarters inches long, consisting of mucus and eosinophile cells. Curschmann's spirals were also present.

During the next two or three days the character of the sputum changes much. It is often small in amount and mucopurulent, with, however, some clear frothy fluid. As a rule, now no Curschmann's spirals are found, although in one case, in which they had been present in good numbers, they were more beautiful than

before. Fibrin casts of the bronchi are sometimes present, occurring with the spirals, which they may exceed in numbers. At the tip of some of the branches the cast may be continuous with the central fibre of a typical Curschmann spiral. Along with the spirals occur large numbers of eosinophile leucocytes. In some cases, but rarely, Mastzellen. Where the eosinophile cells are increased it is common also to find the Charcot-Leyden crystals. Calcium oxalate crystals have also been found. As a rule, the sputum ceases as soon as the attack is well over. In some cases, however, it is almost continuous, even 100 cc. per day, but may have sputum-free intervals.

Curschmann's Spirals.—These beautiful structures occur at some time in perhaps every case of true bronchial asthma. This, however, does not mean that they will be found in every paroxysm of this disease in which they are sought. We have in mind one case, a man whose sputum several years ago furnished the students with the most beautiful spirals. He has since then been admitted during the past fifteen years fourteen times in acute attacks of asthma. Only on one day of this period was a spiral found. While they may be present

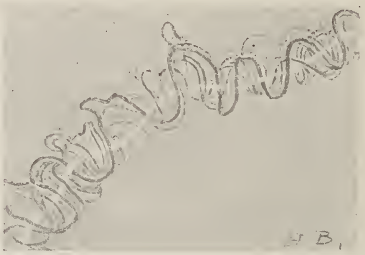


Fig. 17.—Curschmann's spiral, from the sputum of a case of asthma. × 200.

during the paroxysm, they are found particularly just at the end, as the sputum increases, and, as a rule, are absent after it has become mucopurulent. They may be present in large numbers.

In general, two forms may be described. The first is a spirally twisted strand of mucus enclosing leucocytes, eosinophiles, and Charcot-Leyden crystals. The second and more beautiful form consists of a tight skein of mucus wound around a central fibre. This may be from 1 to 2 cm. or more long and 1 mm. broad. It may be branched. These spirals have two parts; the "mantle," which is the mucus surrounding the "central fibre." The mantle contains, besides eosinophiles, many pigmented epithelial cells, some ciliated cells, and Charcot-Leyden crystals. The arrangement of these cells, not mixed, but in lines and groups, is interesting. The central fibre, which probably consists of transformed mucus, is very refractive. It is a spirally twisted strand, homogeneous, with a sharp contour or saw-edged. While the caliber varies, it is quite constant throughout one spiral. Central fibres are subdivided into the small size, from 0.5 to 1 micron in diameter, the medium-sized, 3 microns, and the thickest, even 18

microns in diameter. In sputum which was hardened *en masse* and cut in sections they were found by Ruge to be solid without evidence of the lumen which others have claimed. These fibres are sometimes well developed, sometimes present only as a trace; they may be absent. They end sometimes as a thread, while in other spirals they give off a multitude of lateral threads. The finer are often branches of larger threads, or the smaller may unite to form a larger. Some of the larger types give off fine threads radially to the mantle in all directions. In structure some are lamellated, while others seem to be a bundle of parallel threads, spirally twisted. These central fibres may be differentiated by staining, and hence are not optical phenomena presented by the most compressed part of the spiral, as some claim. They occur alone sometimes (see Fig. 18), and have the same significance as per-

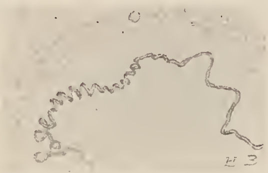


FIG. 18.—Free central fibre of a Curschmann's spiral, from the sputum of a case of asthma. × 200.

fect spirals. When alone they are spirally twisted. In some cases occur perfect spirals; in others, many free central fibres; again others, free central fibres, and some with very imperfect mantles (see Fig. 17).

As regards the origin of the spirals, the central fibres are certainly not casts of the smaller bronchi, since they are often only about one-tenth as big. Schmidt claimed that for their formation the epithelium must be well preserved, that they consisted of mucus secreted in the smaller bronchi, the centre representing the most twisted part, that is, of the greatest relative compactness; and for their formation a tough mucus was the important thing. Hoffmann claimed that the smaller bronchi are themselves spirals, which become straightened out as the lung expands, and that the tough mucus forced through these spirals can assume a spiral shape. Others claim that the cilia motion in the bronchi must be in spiral waves; others that the spiral is formed from a straight band of mucus which in passing the bifurcation of two bronchi is whipped into a spiral by the cilia of the other bronchus, the direction of whose cilia motion will be tangential to the axis of this thread. Gerlach gave three conditions necessary for their production,—a small amount of very viscid sputum, very forcible respiratory movements, and clear bronchi. These three conditions are best given in asthma. He claimed that the mantle and central fibre are formed in the same place, but that the latter is formed later and is merely an optical expression for that part of the mucous mass which has been twisted the most. We would say, however, from studying the spirals we have seen, that while the central fibre is itself spirally twisted, there are many fewer revolutions per unit of length than in the mantle.

In cases which we recently had a chance to study the spirals were beautiful, about 2.5 cm. long and 1 mm. broad, and with a central thread so refractive that it could be definitely seen by the naked eye. While fresh it is interesting that this thread could not be studied with the higher powers because of its high refractivity,

and seemed to be merely an optical phenomenon. At certain points, however, where the mantle was thin and particularly after the spirals were allowed to dry somewhat, it could be easily studied, and was found in some cases to be a bundle of twisted fibres. The mantle was very tightly twisted, and on cross-section at certain points it could be seen that it consisted of spirally wound sheets of mucus reaching from the central thread to the periphery. It was filled with various cells; among these, squamous epithelial cells, very many alveolar cells, many containing coal pigment, and others modified hæmoglobin. In all fields were a great number of eosinophiles, in many, cylindrical cells which appeared ciliated, although the cilia were not as distinct as might be desired, and among them goblet-cells. It is interesting that in the structure of the spiral these cells were not mingled, but each variety occurred in groups or lines and large numbers in each group. The Charcot-Leyden crystals occur singly or in clumps, some quite large. One was projecting from an eosinophile cell and surrounded by its detritus. In some fields full of leucocytes search was made in vain for one which was not an eosinophile. In one specimen was found a strip of mucosa of cylindrical epithelium. Other spirals had no core, and some spirals consisted merely of a very large refractile central fibre. One spiral was particularly interesting; two separate strands of cell-rich mucus, discrete at first, became twisted, the one within the other, into a spiral, and yet each could be traced separately for a time until the coil was so tight they could not be distinguished. The inner was thick, the outer thin, and at the end where they were not spirally twisted their structure could be seen, a band of mucus with detritus and cells in lines; almost all the leucocytes were eosinophilic. If the spirals be allowed to dry, the central thread is much more distinct. In some cases they are a bundle of longitudinal fibres slightly twisted. This case was a typical one, with spirals, immense numbers of eosinophile cells, and a large number of epithelial cells, the alveolar with the various forms of pigment, and the cylindrical, both ciliated and goblet.

In another case were many central fibres without mantles, and some with a few fibres wound around them. These fibres, forming these imperfect, loose mantles, were remarkably thread-like and of quite uniform diameter.

Charcot-Leyden Crystals.—These Charcot-Leyden crystals (see page 34), present wherever the eosinophile cells are increased, are very common in asthmatic sputum. Their number may greatly increase as the sputum stands. These crystals occur in groups, forming specks of a greenish-yellow color, which masses may be even seen with the naked eye. They may give to the spiral a yellowish-green color macroscopically when present in large clumps along the spiral. It is important to bear in mind that their size varies so that their presence can be excluded only when a search has been made with the oil-immersion lens. They increase in size and number as the attack lasts. In cases in which none were found at first they will appear in the sputum if it be kept in a warm chamber. Concerning their composition all that can be said is that there exists in the sputum a substance which after expectoration is crystallizable in this form. The same is true of tyrosin, and the French seem eager to identify the two. They practically always occur in asthma, but careful search is necessary, and in case they are not found it is well to let the sputum stand.

The alveolar epithelial cells laden with golden-yellow pigment may occur in large numbers and fill a considerable part of the mantle of some of the spirals. They occur chiefly in clumps packed

together, and in other parts of the specimen none will be found. It is interesting to see what large masses of these cells will occur in certain parts of the spirals. Their origin, v. Noorden says, is clear; that in asthma we have frequently small traces of blood in the sputum, and that this is the source of the pigment of these phagocytes. These cells are similar to the Herzfehlerzellen seen in chronic passive congestion.

There is a well-marked group of cases which may represent a transitional stage between asthma and fibrinous bronchitis. The sputum contains spirals, Charcot-Leyden crystals, eosinophile cells, all the constituents found in asthma, but also casts of the smaller bronchi, which, however, do not branch much and which may at one end tail off into the central fibre of a true spiral. In one case of Dr. Osler's,[28] already mentioned, these casts were 1 to 3 cm. long.

Acute Bronchitis.—The sputum in acute bronchitis is at the onset very scanty, or even absent. When present this "sputum crudum" is usually tenacious, viscid, very hard to expectorate, and of a frothy transparent appearance. It consists of almost pure mucin. Microscopically a few leucocytes and red blood-cells can be found, also a few bronchial epithelial cells, some ciliated and some with the cilia in motion. There are also a few mononuclear leucocytes, the so-called "mucous corpuscles" which are derived from the lymphatic masses along the tract. In certain conditions so many of these epithelial cells are present that the term "desquamatory bronchial catarrh" was applied. Myelin drops are present, but not very many and only the simpler forms. Such sputum is the result of an increased secretion of the mucous glands, together with the desquamation of a few epithelial cells. As a rule, this clear sputum is present for about two days, yet during the whole course of the acute bronchitis the sputum may represent only a hypersecretion, and hence present the above character. In some of our cases it was two weeks before very much pus was present. In some cases the sputum at this stage is less viscid, hence Biermer's adjective, "seromucous."

After the first two days or more the cough usually "loosens." The sputum is increased in amount, less viscid and less tenacious, and may be like the white of an egg in appearance, since it is frothy and shows whitish streaks. Sometimes blood-streaked, in other cases there is considerable blood at the beginning of the attack; such was true in 33 per cent. of our cases.

The sputum now becomes mucopurulent. It contains all of the above elements, but the pus-cells are very much increased. These may be uniformly distributed, hence the sputum has a uniform yellow color, or may be present in purulent islands. There are still many epithelial cells present, but these have lost their shape and their cilia, are now

[28] See Bettmann, Amer. Jour. Med. Sci., February, 1902.

round, and often fatty. Such sputum was formerly called "sputum coctum." In a typical case the sputum then becomes almost purulent, the pus being poured from the inflamed and probably partially denuded mucosa. This sputum is opaque yellow or a yellowish-green, and is often expectorated in masses. The amount is, as a rule, from 100 to 200 cc. in twenty-four hours. Most is expectorated in the morning. Microscopically, it is found to contain much mucus and much myelin. There are no cylindrical cells now. There are usually some red blood-corpuscles, but the leucocytes predominate in the field, and are chiefly polymorphonuclears, although a certain number of mononuclears are found. Alveolar epithelial cells, some containing pigment and some fat granules, may be found if searched for. Fat is also present in larger masses, which in shape resemble a cell, although now neither nucleus nor protoplasm can be demonstrated. The sputum of some cases is characterized by the abundance of fat, present in cells, in droplets, and in the above-mentioned masses of droplets, while in other cases but little is found. The reason for this difference is not known (Hoffmann). Bierman divided the purulent sputa into three classes. His division has been severely criticised, although the nummular variety was present in a few of our cases. With improvement the sputum becomes more abundant, more purulent, and less tenacious. It then, as improvement continues, diminishes in amount and finally disappears. The above is a quite typical sequence. The following varieties, however, occur. In 13 per cent. of our cases in which the diagnosis of acute bronchitis was made because of the physical signs on auscultation, no sputum was at any time to be obtained. In some cases so tenacious was it that it could not be expectorated, the patient often vomiting in the attempt. In other cases the sputum is mucopurulent and fairly abundant from the very onset. It is interesting, however, that in a large number of these last cases it is probable there was a slight chronic bronchitis already present, since over 50 per cent. of these patients stated in their history that they were subject to coughs and colds. This was true in less than 20 per cent. of those in which the sputum at the onset was of small amount. In general it seems true that a large amount of sputum means a chronic trouble. In about 35 per cent. of our cases the sputum was viscid and very tenacious and scanty throughout the whole course, the patient suffering from a dry cough for a few days after the disappearance of the sputum. Many cases have at the end of the attack a common sputum, not mucopurulent, but consisting of a watery serum in which swim islands of pus which are globules about 1 cm. in diameter, consisting of mucus loaded with pus-cells. Such a sputum on standing will separate into two layers, the upper watery and transparent, the lower purulent. This was true in 10 per cent. of our cases. Other cases were interesting in

that the sputum at the end of the attack became again as at the beginning of a pure mucous type. In acute bronchitis much valuable information may be obtained from the sputum, since it is the best index of that which occurs within the chest.

In the so-called *capillary bronchitis*, that is, acute bronchitis of the smaller tubes, the cough is frequent, often paroxysmal, and at first dry. It may remain so, the sputum being absent throughout the entire course, or expectorated in small quantities with great difficulty. In these cases a diminution in viscosity is a sign of improvement.

Chemical Analysis.—The chemistry of the sputum in this disease has a very slight interest. Of the cases which have been reported by Bamberger, Biermer, and Renk, the water has varied from 95.62 to 98.3 per cent.; the organic substances, from 1.17 to 3.7 per cent., while the inorganic, from 0.457 to 0.76 per cent.

Chronic Bronchitis.—Under this heading may be included all cases from the simple subacute, in which case a cough has merely "held on" for several months, to those cases which give a history of slight cough with expectoration extending over twenty-five or more years. Among the cases which may be considered subacute we have those in which for several weeks or months the sputum is tenacious, viscid, and very small in amount. The patients describe this as consisting of thick leathery lumps; in other cases, as a white sticky mucus. Later on it is apt to become more and more abundant and mucopurulent, and hence yellower. Some sputa have a dark greenish color and a foul odor which will last for weeks. Such sputum as exemplified in our cases is abundant, and will separate to a certain degree into three layers,—a mucous layer, brownish-gray serum, and a mucopurulent sediment. The sputum in these cases will gradually diminish leaving the patient apparently well, but certainly more susceptible to another acute attack.

The acute exacerbations of a very chronic bronchitis form no small part of the admissions for acute bronchitis in a general hospital. These exacerbations may turn a dry cough to one with sputum, or a chronic expectoration of slimy mucus to an abundant mucopurulent sputum, often blood-streaked.

During the acute exacerbations it varies much in appearance. Sometimes it is small in amount, very tenacious and purulent, sometimes of large amount, mucopurulent and slightly tenacious, and in still other cases, and perhaps most common, is an abundant white frothy seromucous sputum containing very little pus. The odor is sometimes foul, and in one of our cases almost putrid. The amount may vary from 100 to 200 cc. in twenty-four hours. Later the sputum increases in amount and presents mucopurulent flakes, sometimes very small. It separates into two layers, with the serum above and the solid particles below, while other cases will have a tenacious green mucus upper layer and a fluid lower layer. Microscopically are found pus, epithelium, and red blood-cells. The most common type of chronic bronchitis is the so-called winter cough, the patient during the winter suffering from cough and expectoration from which he is free during the summer.

Such may be the history for fully twenty years. Later, however, the tendency is for the trouble to be continuous throughout the year. Such cases, as a rule, expectorate only in the morning, and describe themselves as then "clear" for the day. Some such cases expectorate about an ounce of mucopurulent sputum, while in others it is in thick yellowish masses. In severe cases the cough is paroxysmal and the sputum a sticky, frothy phlegm, sometimes blood-streaked, and very hard to expectorate. During the acute exacerbations it is apt to become still more scanty and tenacious. In general, these cases feel best when the sputum is moderate in amount and worse if diminished or increased. Microscopically, it contains much mucus, few pus-cells except in the purulent variety, and much myelin. In some cases it is profuse, mucopurulent, the pus being present in nummular masses of a yellowish-green color which float in a liquid serum, and hence it separates into two layers. In other cases it may also be very large in amount but homogeneous and extremely viscid, filling the cup with a single purulent glutinous jelly-like mass.

Dry Catarrh.—The "catarrhe sec," in the sense of Laennec, is a disputed symptom-complex, but a chronic bronchitis with very little or no sputum is not at all unusual. According to the English, such occurs particularly in "gouty" patients. We associate it, however, more with emphysema and myocarditis. Some of these cases will deny any sputum whatever; in other cases it is glutinous and pearly.

The chronic bronchitis of emphysema deserves especial mention, since it is such a common form. For instance, of 100 of our cases of chronic bronchitis, in 43 per cent. the emphysema was a marked clinical feature. Of 100 cases of emphysema 58 per cent. suffered also from bronchitis, and 47 per cent. from chronic bronchitis. Of the cases with chronic bronchitis, in 11 per cent. it was the dry form, the patient denying any expectoration whatever. In the cases with a slight sputum the expectoration occurred for years only in the morning, and for the most part consisted of a slight amount of bluish-white mucus. It may, however, be large in amount. One case, for instance, for years was awakened at five o'clock each morning with a severe paroxysm of coughing and expectorated large amounts of thick mucus, in an almost solid mass. In other cases the sputum is abundant, whitish in color, frothy, and about one pint a day. In our cases of emphysema with chronic bronchitis admitted with an acute exacerbation the sputum varied, being in some cases in very gelatinous, opaque masses, and in one-fifth of the cases blood-streaked. The small amount of sputum became a large amount, sometimes of white frothy mucus, at other times of blood-stained mucus and pus, in other cases it approached blood-stained serum and in one case was putrid. As the cases improved it became still more abundant, white and frothy with very little pus, and then gradually diminished to the previous state. Among these sputa of chronic bronchitis a few may be mentioned in particular. In two a great many eosinophile cells were present. Some were remarkable because of the large amount of myelin, and in others large masses of fat globules were very noticeable. In one case of simple chronic bronchitis the man had had for ten to twelve years a slight expectoration. On admission the sputum was thin, cloudy, and abundant, consisting of serum in which was a sediment containing moulds of the bronchi from medium size to 0.5 mm. in diameter, and consisting of mucus with pus and alveolar epithelial cells. The sputum also contained much pigmented alveolar epithelium, pus-cells, and red blood-cells. In a case of chronic bronchitis in an emphysematous person during a rather acute attack with a steadily elevated temperature the sputum was found to be considerable in amount, seromucoid, and never bloody in appearance, and contained during repeated examinations large numbers of sarcinæ. In one case of chronic bronchitis with emphysema and "hay fever" the sputum was yellowish-green, mucopurulent, slightly blood-tinged, containing branched plugs

of the bronchi which consisted of mucus and pus in which were many eosinophile cells and masses of the mycelial threads of some mould.

In the chronic bronchitis of cardiac disease, especially mitral, the sputum is characterized by the large amount of blood which may be present. This may be fresh or changed enough to give a prune-juice appearance, and in cases, particularly of mitral stenosis, may be grossly stained by the large numbers of Herzfehlerzellen which are constantly present. In other cases there is a daily large amount of frothy seromucous pus.

Bronchorrhœa.—If by bronchorrhœa one means, with Laennec, a chronic idiopathic disease, the existence of this form is exceedingly doubtful. But if by the term is meant a chronic bronchitis with an abundant sputum, it is by no means rare. There is one form described, no example of which we have had in this hospital, of a "bronchorrhœa serosa," or "asthma humidum," with an abundant, very watery, colorless, foamy sputum. Some of these cases are said to have a neurotic basis. In the bronchorrhœa of chronic bronchitis the sputum may be very large in amount; commonly it is purulent, watery, of a green or a yellowish-green color, and in amount about 500 cc. a day. In these cases of "bronchoblennorrhœa" the bronchi have been denuded of mucosa and are lined by a pyogenic membrane, hence little mucus is secreted and the sputum is a profuse watery pus which separates easily in three layers and which may have a very bad odor, although not distinctly fetid. Such sputum is seen also in bronchiectasis, and perhaps in cases of putrid bronchitis and lung gangrene.

Putrid Bronchitis.—In some cases of chronic bronchitis there is a disagreeable almost fetid odor to the sputum, but in putrid bronchitis a truly fetid expectoration is present. This occurs with most cases of bronchiectasis, gangrene of the lung, abscess, those in which the sputum decomposes within tuberculous cavities, and in empyema perforating through the lung. A true simple bronchitis without dilated tubules and yet with a fetid expectoration is certainly very rare (Fowler and Godley), while some deny that it ever exists (Hoffmann), and claim that the most of the cases thus catalogued are probably of bronchiectasis. A case of putrid bronchitis would quite surely result soon in dilatation of the bronchi, and a case of bronchiectasis very often soon has a fetid expectoration. A very few genuine cases have, however, come to autopsy (Osler).

The sputum is an abundant, profuse, watery pus, of a dirty ashy-gray or a brownish color, and with a horrible odor which sometimes will fill the whole house. Allowed to stand, it separates into three layers,—the upper of frothy air-containing mucus, usually small in amount, since the mucous membrane is for the most part destroyed and replaced by a pyogenic membrane, and hence secretes little mucus;

from this layer extend downward brownish strands. The middle layer is of serum, while the lowest is a thick sediment of epithelial cells, fatty cells, free fat, almost pure pus, all kinds of bacteria, and sometimes Dittrich's plugs. No elastic tissue or fragments of lung are to be found, which excludes gangrene, which may, however, result. Chemically are found many of the products of decomposition of proteids, volatile acids, among them butyric, valeric, and others; NH_3, H_2S, leucin, tyrosin, etc.

Fibrinous, Croupous, or Plastic Bronchitis.—By this term we here mean the chronic, idiopathic form, not the acute form occurring in the course of certain infectious fevers (see page 24). This chronic form is a very rare disease as is shown by the fact that Bettman was able in the literature of thirty-five years to find only twenty-seven cases. It is very little understood. The sputum in such cases is catarrhal, consisting of abundant mucus for about five or ten days, and then after a severe coughing spell the expectoration of a bronchial cast. In some cases the amount is considerable, even more than one pint per day. Blood is quite often present in the sputum, either before or after the expectoration of the cast, but generally with it, and yet true hemorrhage is rare. The casts are formed and expectorated at various intervals, usually with intervals of months, sometimes as often as one in from one to three days, and in one case three in one day. They are seen as formless masses in the sputum. After shaking them out in water they are found to be moulds of a bronchial tree. Those from the same case will often present exactly the same shape as if they were all from the same lobe. Sometimes they will appear to represent the tree of a whole lobe. The size of the largest of them is about 10 cm. long. They are grayish-white in color, contain a great many air-bubbles, and in about one-third of the cases are blood-streaked or contain a clot in the centre. On cross-section they are found to consist of concentric layers, apparent either grossly or microscopically; the inner layer presents many whorls, since this layer, the oldest, has been telescoped into those more recently formed. The casts are usually hollow, although some are solid; others are hollow in the larger branches and solid in the smaller, others *vice versa*. In the central layer, the oldest, are seen the remains of many cells, alveolar and bronchial epithelium, leucocytes, red blood-cells and bacteria. Sometimes there is much fat in the casts and in the sputum.

Casts do not always arise from nor are they produced by the epithelial cells of the mucosa, since in the above-mentioned case in which three were expectorated in one day, there was found to be no epithelium in that part of the bronchial tree. These were therefore a direct exudation. They were formerly supposed to consist of fibrin, since physically the material resembled this. Others claim that they are of

mucus, one says syntonin, another coagulated albumin, because of the chemical reactions. Some portions take Weigert's fibrin stain, but the most of it does not, hence it may be said that their composition is rather uncertain. Liebermeister [29] reviews the question at length as the result of the study of one fresh case and twelve museum specimens. He found fibrin and mucin present in seven of the thirteen cases. Weigert's fibrin stain cannot be trusted in these cases. For fibrin he prefers Kockel's method, and thionin for the mucin. In fibrinous bronchitis the cast is of a loose texture containing much air, almost fills the lumen, and contains few cells. In diphtheria of the bronchi the cast consists of a firm hollow membrane of dense fibrin strands with countless cells. A cast from a heart case at death was similar to those of fibrinous bronchitis.

Charcot-Leyden crystals are commonly present in the cast. In the same sputum sometimes spirals are found. In Dr. Osler's case, mentioned by Bettman, the ends of some branches of the casts were directly continuous with the central threads of true spirals. Many eosinophile cells are sometimes found, also red blood-cells, hæmatoidin crystals, and lecithin granules. In Vierordt's case [30] there were many such casts, and on one occasion a typical Curschmann spiral.

Bronchiectasis.—The sputum in the saccular form of this disease is often very characteristic; in the diffuse form not at all. In the former it is marked by two features,—its profuseness and the periodicity with which it is expectorated. This periodic feature was well shown in ten of our twenty-four cases. The expectoration occurs usually in the morning, and depends upon the position of the sac; an irritation of the bronchus due to a discharge of some of the contents of the sac caused by a change in attitude leads to a paroxysm of coughing and hence the emptying of the whole sac. The amount is profuse, as a rule, from 750 to 900 cc. in twenty-four hours, while in one of our cases it frequently exceeded one litre. Such profuse expectoration may extend over a considerable period of time.

In general it may be said that the amount bears no relation to the duration of the disease, for one case of twenty-six years' standing expectorated but from 15 to 30 cc. a day. Nor does it bear any relation to the size of the cavity, as was shown by one of our patients who expectorated more than one litre of sputum a day and yet at autopsy a few surprisingly small cavities were found. Of twenty-three cases, in two the sputum for twenty-four hours was under 100 cc.; in eleven, from 1 to 300; in two, about 500; while in seven, over 600 cc. It is stated that the diminution in amount as the patient grows weak before death is surprising.

The most characteristic sputum is grayish or grayish-brown in color, fluid, purulent, of a disagreeable odor, and separates into three

[29] Deutsch. Arch. f. klin. Med., 1904, Bd. 80, 5 and 6.
[30] Berl. klin. Wochenschr., July 16, 1883.

layers on standing. This character, however, is by no means constant. A bronchiectatic cavity lined by mucous membrane will before infection secrete a pure clear mucus, but after infection has occurred, as is the rule, the mucosa is soon reduced to a pyogenic membrane which secretes a yellow purulent fluid with a sweetish odor. This may last for years. Sooner or later putrefactive changes may set in. The sputum is then mucopurulent, and of any shade of gray or green; those with the worst odor in our cases were of a dirty gray color. If blood be present the color will present different shades of red or brown according to the chemical changes in the hæmoglobin. While as a rule it is very fluid and watery, in some cases it is thick and viscid, while in other cases the sputum is mucopurulent and contains masses suggesting nummulæ. In other cases, as was shown in our series, particularly those improving under treatment, while it was profuse and watery at first, it later diminished in amount, was mucopurulent, and of a less offensive odor. The tendency to form three layers on standing in a tall glass vessel was marked in fourteen cases. These layers are: an upper frothy mucous layer, a middle serous, and a lower granular layer. From the upper often hang down through the fluid strands or "streamers," as they are sometimes called, of the same material. Hoffmann and others mention but two layers, omitting the upper mucous, which was absent in three of our cases. The lowest layer is always thick.

In four of our cases there were four well-marked layers; the lowest of an abundant, greenish-red, purulent material; the one above containing a good deal of blood, and hence was red or brown; over this a serous layer, while on top a frothy mucous layer. In other cases below the frothy mucous layer was a mucopurulent layer with streamers hanging down through the fluid and which with a little encouragement would probably all have sunk. The odor is, in general, bad, but in two of our cases it was not at all offensive. In some cases there is at first none, then a slightly offensive odor of a heavy, sweet nature, while after the putrefactive changes have set in it will be of a fetid character. These changes are due to secondary infections of the contents of the cavity. In ten of our cases the odor was heavy and sweet, while in ten others it was at some time very fetid. This is not exactly the same odor as in gangrene, but has been described in some cases as "pseudo-gangrenous," resembling the odor of rotten cabbage, or garlic. This odor will often diminish after creosote inhalations, or intratracheal injections, and a patient admitted with extremely fetid sputum may leave the hospital with a much reduced sputum not at all offensive. The breath is sometimes worse than the sputum. The odor is largely due to H_2S, NH_3, and various volatile acids, among which are acetic, butyric, and formic.

Hemorrhages into the cavity are common. Some put the figure at 50 per cent. They occurred in seventeen of our twenty-four cases. While slight, as a rule (eight cases), it is sometimes considerable (six cases), while in three of our cases it was extreme. In other cases it is fatal.

One of our cases, a man, was admitted to the hospital fourteen times, and five times because of extreme hemorrhage which threatened his life. At one of these admissions, in the course of a very few days he had six large and several small hemorrhages, reducing his blood rapidly from about normal to 1,090,000 red blood-cells with 20 per cent. of hæmoglobin. In another case on one day 1700 cc. of blood were lost in about ten minutes. Another smaller hemorrhage the next day was fatal.

Microscopical Constituents.—The cavity walls of an uninfected case secrete mucus. If the outlet be closed, the cavity will contain mucus with desquamated epithelial cells. After infection, however, the constituents are those of an abscess, since soon there is no mucosa and the walls are merely pyogenic membrane; after the fetid infection the elements are those of fetid bronchitis. The pus-cells, enormous in numbers, are well preserved, fatty, or vacuolated. The red blood-cells are unchanged or very much altered. It is rare to find elastic tissue, which would mean ulceration of the walls and was present in two of our cases. The fatty acid crystals occur especially when the outlet of the cavity is small, thus allowing considerable stagnation. These crystals are often very large in size, numerous, and present a beautiful picture. They were abundant in four of our cases (see Fig. 6.) Cholesterin occurs; hæmatoidin crystals, leucin, and tyrosin, sometimes; Dittrich's plugs very commonly. The alveolar epithelial cells are usually present, containing pigment and, in some cases, much myelin or fat. No tubercle bacilli are found, but bacteria in great numbers in large zooglia. Yeasts occur, and in one of our cases a definite aspergillus mould was found. In the contents of these cavities calcium salts are sometimes deposited, giving rise either to a clay-like mass or to the so-called "lung-stones." In two of our cases these lung-stones were present, and in one of them, a man who had expectorated several, at autopsy considerable calcareous concretion was found embedded in the walls of a cavity. The stones in the sputum were about the size of a split pea.

In other cases, as in thirteen of our series, the sputum is by no means so characteristic, but presents all the characters of a chronic or a fetid bronchitis.

In the bronchiectasis of children it is important to remember that all the sputum may be swallowed and vomited.

Gangrene of the Lung.—In this disease the sputum most characteristic is profuse, extremely fetid in odor, of a greenish-brown color, separates easily into layers, and contains shreds of tissue. The latter point alone differentiates it from putrid bronchitis. Its odor is the worst of all, yet in some there is none whatever and no fetor of the breath. In five of our twelve cases the presence of gangrene was unsuspected, one case expectorating merely "phlegm." This odorless sputum is seen particularly in diabetics (in one of our cases it was an

autopsy surprise), in the insane, and in gangrene from embolism in which case it is localized and the infected area not yet discharging into a bronchus. In cases which recover the odor gradually disappears. In nature the sputum is profuse, thin, frothy, watery, of a dirty greenish-brown or ashy-gray color. The color varies considerably. Those which contain little blood are of a dirty gray or greenish tinge, while those which contain blood will vary from reddish-brown to brownish-red according to the degree to which the hæmoglobin is changed. Other sputa are described as chocolate in color. The sputum separates easily into three layers,—the upper of frothy mucus, the middle of serum, and the lowest, always a large one, of pus, tissue detritus, Dittrich's plugs, and tissue fragments. From the top layer streamers often extend down through the fluid. In other cases the sputum is mucopurulent in nature. It may be viscid, lumpy, mixed with blood, and yet very fetid.

Macroscopically of chief interest are the fragments of necrotic lung tissue. These vary from those most minute to fragments several centimetres long, of a sooty appearance, with ragged outline, or surrounded by a grayish-yellow mass. These fragments are of firm tissue, or of colorless ground substance full of granular detritus or fat droplets, clumps of coal, large fat needles, bacteria, and elastic tissue. Dittrich's plugs are also found. The other constituents of the sputum are those of fetid bronchitis. There has been considerable dispute whether the presence of elastic tissue has any diagnostic importance. Some claim that it is rarely if ever present in this disease, and think it is digested by a ferment. Osler says that he has never seen a case in which it was absent. Dittrich's plugs were present in some of our cases, but Osler considers them rare. Alveolar epithelium, often pigmented, occurs; fatty acid crystals and fat droplets, often many; cholesterin, leucin, tyrosin may be present; masses of bacteria and of leptothrix occur, while flagellata have been described. In one case mentioned by Sahli, in which the infected area was non-odorous, large numbers of sarcinæ were found. Blood is frequently present. This hemorrhage is principally from the opening of very small vessels, not by diapedesis. Fresh blood was present in five of our cases, but as a rule it is much altered and the hæmoglobin present as methæmoglobin and hæmatin.

Many have found acid-resisting organisms in the sputum in such cases (see page 51), Mayer in ten of fifty-eight cases, also several others; organisms probably to be classed as actinomyces (streptothrices), which seem related to the timothy grass, the butter, *et al.*, bacilli. These are not alcohol-fast.

Abscess of the Lung.—In abscess of the lung the most characteristic feature of the sputum is the sudden appearance of a large amount

of quite pure pus in which are fragments of lung tissue. It may be many hundred cubic centimetres in amount. If allowed to stand it will present a certain layer formation, but not a characteristic, since a slight shaking will restore its previous homogeneity. Its odor is at first faintly sweet like all pus, but since gangrene supervenes often, it then becomes foul, yet not as foul as that of gangrene and putrid bronchitis. The tissue fragments are of particular importance. These are pieces of lung tissue permeated by pus-cells which give them a yellowish-gray color. In size they vary from about a millet-seed to fragments even two inches long. They consist of a framework of elastic tissue, the remains of blood-vessels, masses of coal-dust, fat crystals, free fat, detritus, hæmatoidin crystals, amorphous clumps of pigment, and zooglea of cocci. In other cases there is a so-called "insensible disintegration" (Leyden) of the lung without the appearance of any large fragments. In such cases separate elastic fibres will be found. The other microscopical constituents are free elastic fibres, cholesterin, fatty acid crystals, free fat, lung pigment, detritus, hæmatoidin crystals, which may be present in large numbers and give to the whole mass of sputum a brown color, and bacteria. In the text-books, particularly of older writers, has been described the gross appearance of a sputum which escapes from a large cavity slowly through a small opening. In this case the pus as it escapes in a thin thread receives a mucous coating which prevents its coalescence. Hence the sputum when shaken out in water will appear like a skein or thread of pus. In our cases there has been no such appearance.

The sputum of a LIVER ABSCESS perforating through the lung, which sometimes causes also a lung abscess, in other cases an hepaticobronchial fistula without a local abscess, is often characteristic. Grossly it presents a so-called "anchovy-sauce" appearance, or the tint may be ochre-yellow due to the bile. The patient will complain of the bitter taste due to the bile acids. Microscopically bilirubin crystals and much elastic tissue will be found.

In our records there is a series of seven such cases. In three the sputum was abundant, exceeding even a litre in twenty-four hours. Expectoration may be paroxysmal, even a quart at a time. The odor was mildly offensive in two, and more so in two others. Six of these cases presented the typical anchovy-sauce appearance of a rusty-brownish-red color, and frothy. In four cases the sputum was at times distinctly blood-streaked, in two purulent. Microscopically may be found the ordinary elements of sputum, pus, red blood-cells and alveolar epithelial cells. In addition the hæmatoidin (bilirubin) crystals or needles may be a marked feature, as was true of two cases. The elastic tissue was found in considerable amounts in five cases, and at certain times the quantity may be very great. Fat crystals are present. In two cases the liver cells, it was thought, could be recognized. The living active amœbæ were found in five cases, in one long before they could be found in the stools even after repeated examinations. It is interesting to note how often the sputum which contains the amœbæ will also contain much

elastic tissue. If the sputum be preserved in the thermostat, they will remain alive and motile for a day or so.

Abscess of the lung followed acute lobar pneumonia; six cases. In three of these there were no clinical features which would suggest this discovery at autopsy. In these cases the abscesses were small and multiple, and before death there was little or no sputum. In one case in which the diagnosis was not made the only change in the sputum was that the viscid tenacious blood-streaked expectoration became less tenacious. In one case a small amount of a very tenacious blood-tinged sputum became suddenly very dark, of a brownish-black color, mucopurulent, and then greenish and small in amount. It then disappeared, soon to reappear as a mucopurulent, very green, scanty sputum, and soon became large in amount, very thick, very purulent, and of a sour odor. It then became thinner, more watery, but blood-stained, containing elastic tissue. Then it reduced in amount, became mucopurulent, and finally, with the recovery of the case, ceased. In one case large numbers of trichomonads were in the sputum.

Three cases of post-operative abscess were followed clinically; one was admitted with a paroxysmal cough and the sudden expectoration of a foul-tasting sputum, which later became sweetish and of a less disagreeable odor, yet we thought bad enough. The expectoration was large in amount, and contained pus and fatty acid crystals. There were large fragments evidently of tissue, even 5 by 3 cm. in size, but so decomposed that the structure could not be well made out. The sputum then became less profuse, mucopurulent, and the patient recovered. In another case the sputum was very foul and contained much fat, while in the last case it was large in amount, foul, purulent, and blood-streaked. Of two other cases, in one the sputum did increase, but the diagnosis was not made, while in the other an abundant blood-streaked, brownish sputum of no especial odor suddenly increased in amount, became dirty, frothy, and foul, slightly streaked with blood, and separated easily into three layers. At autopsy a large abscess cavity was found.

Perforating Empyema.—The sputum of these cases resembles abscess of the lung, with the exception that there is less elastic tissue and practically no tissue fragments. There will be many hæmatoidin and other crystals. The odor, that of pus at first, in some cases described as resembling old cheese, is soon vile because of the infection which commonly follows. In case the pleural fluid escapes slowly through a small opening, it is said that there may be present the fibrillary nature of the pus seen when an abscess is discharged, and due to a coating of mucus around the thread of pus. When the opening is large the pus will escape often rapidly, yet without causing pneumothorax. Allowed to stand, it separates into three layers,—the upper of mucus, the middle of the pus serum, and the lowest of pus-cells.

Perforating Serous Pleurisy.—This is exceedingly rare. The sputum is like that of œdema of the lungs, but contains more albumin, on boiling becoming even solid.

The Serous Sputum of Œdema of the Lungs.—In this case there are large amounts of a frothy, cloudy, colorless, or slightly bloody sputum. If allowed to stand, it presents an upper abundant frothy layer, a foamy fluid, and a lower scanty of pus and the elements of the pre-existing sputum. The most is quite pure serum. The fluid is frothy since it is so rich in albumin, watery since directly from the

blood, and contains only a trace of mucin. Cases presenting this sputum are common enough, and the appearance of this stream from the mouth and nostrils is one of the most gruesome sights of the sick-room.

The Albuminous Expectoration of Thoracentesis.—Of the recent articles, the student is referred to Riesman[31] and Allen.[32] This condition follows a thoracentesis in which the fluid withdrawn has been large in amount and rapidly removed. Terrilon has grouped the cases into three classes. The first is of mild cases, the sputum little to 800 cc.; the condition of the patient is always good. The severe cases are accompanied by dyspnœa and collapse, and an expectoration of from 1200 to 1500 cc. The grave cases are marked by a sudden onset; the fluid may gush from the mouth and the patient die at once from suffocation from the fluid which he cannot expectorate rapidly enough, or, indeed, he may die before he expectorates any. As a sequela of thoracentesis it is rare.

The onset is, as a rule, in less than one hour, or it may come on during aspiration. The latest case began eighteen hours after the tapping, and lasted for twenty-four hours. The duration may be from several hours to a day, but as a rule it is from one to two hours. The fluid is richly albuminous and hence viscid, frothy, and neutral or faintly alkaline in reaction. Chemically it may be tested by heat and nitric acid, or by nitric acid alone, or by potassium ferrocyanide. The sputum should be diluted and filtered and the filtrate tested. Acetic acid gives a precipitate of mucin. It also contains urea, hæmoglobin, and the various salts of blood-serum. Urobilin has been found. The amount is generally from 200 to 900 cc. Two litres have been expectorated. On standing it separates into three layers,—the upper whitish and frothy, the middle opalescent and yellowish or greenish, the lower more viscid, containing a few whitish flocculi, and sometimes slight traces of blood, but rarely much. In Riesman's case there was no lower layer, the specific gravity was 1018, the fluid became solid on heating, the total solids were 5.84 per cent. In Allen's case reported from this clinic the expectoration began in half an hour after 3100 cc. of pleural fluid had been removed, and lasted four hours. It was about one litre in amount, frothy, pale green in color, with a muddy sediment. Microscopically were found flat epithelial cells, a few leucocytes and red blood-cells, and many bacteria. The analyses differ widely. The fluid, while sometimes resembling that of the pleural exudate, in some analyses differs considerably from it. The cause has been much disputed. The majority of writers think that it is due to an acute œdema of the lungs, the result of their rapid ex-

[31] Amer. Jour. Med. Sci., April, 1902, p. 620.
[32] Johns Hopkins Hosp. Bull., January, 1903.

pansion, but the mechanism of which is very much disputed. We would call attention, however, to certain cases occurring during para-thoracentesis and followed by pneumothorax. Some of these cases suggest the expectoration of the pleural exudate, and the demonstration that in many cases the two fluids differ does not disprove the claim that in certain the fluid does come from the pleural cavity.

Hæmoptysis.—For the causes of pulmonary hemorrhage we will give a summary of the chapter on this subject in Osler's text-book.

Hæmoptysis may occur (1) in young healthy persons without known cause and without subsequent symptoms. (2) As the first symptom of pulmonary tuberculosis, or (3) in a well-marked case. During the early stages it is due to mucous erosions and diapedesis; later to the rupture of an aneurism in a branch of the pulmonary artery, which is exposed by cavity formation. (4) Other diseases of the lungs, and this list includes practically all pulmonary disease. Among them are pneumonia at the onset, "bloody bronchitis," cancer, gangrene, abscess, bronchiectasis, tumors, cysts, and actinomycosis. (5) Heart disease, especially mitral. As a rule slight, yet it may be profuse and recur for years. (6) Vascular degeneration, the result of increased pulmonary tension, seen in emphysema and arteriosclerosis. (7) In ulcerations of the larynx, trachea, and bronchi it may be profuse and rapidly fatal. (8) In aneurisms it is sometimes sudden and fatal; in other cases the so-called "weeping" may persist for weeks, or the pressure of the aneurism as a tumor may cause an erosion of the mucosa. (9) An extremely rare form of vicarious hemorrhage due to interrupted menstruation. (10) In rheumatism. (11) Malignant fevers, the so-called hemorrhagic type. (12) Purpura hæmorrhagica and various other blood diseases, among which are hæmophilia, leukæmia, and scurvy. (13) Distomatosis (Westermanii).

The amount of the blood may vary from a mere speck or a few small clots to a quart or more. In general it is of a bright red color even when of venous origin since it is aërated in the lungs, frothy from its admixture with air, and always coughed up. When it clots in the bronchi, casts of these may be formed. In gastric hemorrhage, as a rule the blood is dark, due to the transformed hæmoglobin the result of the action of the acid gastric juice, not frothy, partly coagulated and vomited. Such points are easy enough to determine when the doctor is the observer, but from the history given often difficult, for in their anxiety the friends will not notice such fine points. The severe coughing often causes vomiting, while the coughed blood may be swallowed and vomited. Aspiration of blood from a gastric ulcer will also cause a certain amount of coughing. The gastric blood may be bright if the stomach be empty and a large artery be opened, while the pulmonary blood may be dark and not frothy, provided a

large branch of a pulmonary artery be eroded. A most important point in determining the origin of the blood is the history of the case, whether of previous lung or stomach trouble. Subsequently in a case of hæmoptysis the sputum will for some days be blood-tinged. In case the hemorrhage was from the stomach, there is usually considerable blood in the stools, but a small amount of blood could be explained as that swallowed. It is important to recognize the so-called spurious hæmoptysis, in which case the blood may arise from varicosities of the veins at the back of the tongue or lesions in the throat, glottis, or œsophagus. It is said to be common for young anæmic girls to complain of hemorrhage in the morning which has as its source the spongy gums.

Hæmorrhagic Infarction.—In many cases this diagnosis may be made from the inspection of the sputum alone. This is in discrete masses which remain isolated in the cup, and which appear to be pure blood, but are found to consist of a very tenacious mucus which is intimately mixed with pure fresh blood. In other cases these balls will be of a glairy mucus, with considerable blood-streaking. Expectoration begins at once with the cough and the pain, the character of the previous sputum changing considerably at this time. Such was true of half of our cases. Microscopically, the mucus and the red blood-cells form the most of the mass, and leucocytes are remarkably few in number or even absent, while alveolar cells loaded with blood pigment are usually present in enormous numbers. This, however, may be explained from the fact that these infarctions are particularly common in mitral disease. In other cases the sputum is much less characteristic, as is often seen in cases in which there was considerable previous to the embolism. Sometimes it is a real hemorrhage. Such was true of one-third of our cases. Sometimes the sputum is pneumonic in character. In other cases it resembles the brick-red sputum of chronic passive congestion. In such the diagnosis is said to be hard. In one-fifth of our cases there was practically no sputum. In one, however, the patient remembered some blood-streaked sputum before admission to the hospital.

The above-described character of the expectoration is soon lost, in a very few days sometimes, but usually in about one week the sputum is merely blood-stained and will soon be free from blood. With recovery also it becomes more watery. The amount of blood certainly bears no relation to the size of the infarctions. This was well seen in one of our cases with very large infarctions and only slightly blood-streaked sputum.

Chronic Passive Congestion.—In chronic passive congestion, especially due to mitral disease and particularly stenosis, the sputum is characteristic. The expectoration is chiefly in the early morning, and

consists of a white mucous background, colored by dots or streaks of a rusty color; or the whole mass may be uniformly rusty. These dots, streaks, or uniform tinting are due to the large masses of Herzfehlerzellen; that is, to the alveolar epithelial cells laden with golden yellow granules of amorphous pigment derived from the red blood-cells which have escaped into the alveoli by diapedesis. It is in this condition that the large number and the constant presence of these cells have a great diagnostic importance. This importance was impressed upon us by one case which I will mention in detail. The man spoke only a language for which we could obtain no interpreter; a history of his case was therefore out of the question. His heart was repeatedly examined and reported practically negative. The sputum, however, contained constantly large numbers of Herzfehlerzellen. The pleural exudate was hæmorrhagic and contained mulberry-like masses of the proliferated endothelium of the pleural cavity. He died in a few days without a diagnosis. At autopsy there was found a mitral stenosis of an extreme degree, one of those cases common enough without any heart murmurs, and several large pulmonary infarctions.

Malignant Disease of the Lungs.—The sputum of this has in some cases been described as "characteristically gelatinous, of a red or blackish-red color like currant-jelly," but this is by no means common. In other more common cases it has a prune-juice character. A grass-green or an olive-green sputum has also been found resembling that of caseous pneumonia. A prune-juice sputum (present in ten of eighteen cases) Stokes thought an important sign. In any case a search should always be made for the fragments of the tumor. Our cases presented no important points. In one case of secondary metastasis into the lung, although the area involved was large, there was practically no sputum. In another case of a large tumor the sputum was very viscid, slightly rusty, of a greenish-red color, not fetid, and consisted of pus, red blood-cells, and alveolar epithelium with much myelin degeneration. The next day it was of a dirty grayish mucopurulent character, and at times contained considerable blood. In the case of an epithelioma of the bronchus there was considerable expectoration and several severe hemorrhages. At other times the sputum was seropurulent, liquid, blood-streaked, not tenacious, and frothy. Diagnosis has in several cases been made from the tissue fragments in the sputum.

In mediastinal growths the expectoration is due to the bronchitis resulting from the pressure, and will present the various characters of this condition. If, however, the size of the tumor causes a narrowing of the bronchus, this may lead to bronchiectatic cavities, and a profuse fetid expectoration be the result. Gangrene may supervene.

Syphilis of the Lung.—Fowler and Godley state: "Evidence of excavation with fetid expectoration which does not contain tubercle bacilli should always suggest the possibility of the case being one of pulmonary lues." The expectoration may be profuse, purulent, and offensive, fetor being a common characteristic in advanced cases. With stenosis of the bronchus, a common event in this disease due to the extensive formation of connective tissue at the hilum of the lung, bronchiectatic cavities will form and the sputum present all of the characters of this condition. While hemorrhage is not common, some cases attract attention by the remarkably bloody nature of the sputum. Writers have stated that unless repeated examinations for the tubercle bacilli be made, cases will pass for consumption. Osler, on the other hand, states that he has never seen a case which resembled tuberculosis clinically.

Pneumonoconiosis.—According to the dust which is inhaled this has received various names,—anthracosis, in which case it is coal-dust; siderosis, with the expectoration of iron dust; and chalicosis, in which it is a silicate or other rock-dust. The expectoration is in general muco-purulent, often profuse, and laden with the above-mentioned dusts (see page 21).

CHAPTER II

THE URINE

GENERAL CHARACTERISTICS

The Collection and Preservation of Urine.—It is of the utmost importance in all chemical examination of the urine that a complete and well-mixed twenty-four-hour specimen be obtained, so much do the various voidings of the day differ. To accomplish this most patients need to be watched, and one must rely much on the attention of nurses and orderlies.

In this clinic the day's collection begins at about 6 A.M. The patient then voids, this is thrown away, and the urine then collected until 6 A.M. the next day including this hour's voiding. In case we separate the urine of the day and the night, the former period extends from 6 A.M. to 9 P.M.; the remaining hours are those in which the patient is, as a rule, asleep.

It is very essential that a clean bottle be employed and some means used to prevent the very rapid bacterial action. There is no one preservative which is good in all cases, and the worker should choose his agent with reference to the use to which he expects to put the urine. For instance, for chemical work we usually use chloroform, enough so that several drops remain at the bottom. The bottle must be tightly corked or bacteria will certainly grow in the upper layers from which the chloroform is volatilizing. We prefer this, since it adds nothing to the volume and can be entirely removed. The disadvantages of it are that the formed elements are not well preserved for microscopical examination, although crystals are, and that certain chemical changes do result, whereby, for instance, a suggestive sugar-test with Fehling's can be obtained in urine thus preserved; yet even for oxybutyric acid determination the urine may be kept unchanged even for years. A few crystals of thymol are often used. A slight disadvantage to this is that the urine will give a test similar to bile. Gum camphor is very commonly used. Formalin is of value to preserve microscopical constituents. A person must be wary in a chemical examination of such an urine, since formalin is an active reducing body, and the diagnosis of glycosuria has been made. Other workers employ a dilute chloroform water or a saturated borax solution, adding one-fifth volume to the urine. The disadvantage of these is that they change the volume of the urine and cannot be removed.

Sometimes a twenty-four-hour specimen is not desirable. For instance, in the diagnosis of slight chronic nephritis a comparison of

the urine first voided and that voided at the end of a day's work gives valuable information, also in a suspected case of cyclic albuminuria. Again, in diabetes mellitus of a very mild degree the urine voided three or four hours after a hearty carbohydrate meal may contain sugar sufficient for a positive test, while if this voiding be diluted by mixing it with the whole twenty-four hours' amount the sugar percentage would be too small to be detected. For microscopical examination the urine should be tested as early as possible after voiding, and, if possible, without the addition of any preservative. Formalin is said to add a crystalline component to the sediment (May).

The **value** of urinary diagnosis as a routine practice cannot be too strongly emphasized. About fifteen minutes are sufficient to find out if anything unusual demands further attention. The doctor or student who employs the "X-ray test" or the "sink test" is a traitor, and should be treated as such.

The unexpected is found quite often, and the perfectly healthy appearance of the patient is no guarantee that the urine will not clear up the case. The surgeons especially need this warning. A recent case was a lesson, for one urine examination would have probably prevented an operation following which the woman went into diabetic coma and died.

The Amount of Urine.—The limits of the amount of urine to be considered normal vary widely, both for individuals, and depending upon this, for different countries, especially those in which the customs are fairly uniform. In general it depends on the amount of water in the food and of solids in the blood, especially salts, to be excreted, their excretion increasing the water output. The limits usually given are from 1500 to 2000 cc. That may be true for a country in which beer-drinking is very common; it is, however, too high for others, as for this, where from 900 to 1200 are more common figures. For France the figures 900 to 1500 are given (Becquerel). In women the output is slightly less than in men. The amount of urine also depends on the size of the person; in an adult it is almost directly proportional to his weight. This is not true in the case of children, who excrete relatively more than do adults; newly born infants, from 150 to 200 cc. a day, and children from three to five years of age, about 700 cc.

The amount depends chiefly on the volume of fluids consumed. The extreme physiological limits, depending chiefly upon this, are from 800 to 3000 cc. The increased output reaches its maximum in from two to three hours after drinking a large amount of water, and is over in from five to six hours. Yet, as several have shown and all experienced, the water output is perhaps the most capricious of all the urinary constituents, and the water ingested is only one factor in the question.

The functional limits of the kidney are something enormous, as is seen in diabetes mellitus, in which a practically normal kidney may eliminate 25 litres of urine, an absolutely increased amount of the normal solids, and several hundred grammes of an abnormal solid, sugar, and stand this increased work for some time without any sign of disease. Külz was able in a rabbit by intravenous injection of salt solution to increase the urine to 256 cc. per hour for nine hours, and yet the qualitative composition of the urine remained normal. Insensible and especially copious perspiration affects the amount of urine, which is therefore greater in cool weather than in hot. The latter, however, can be really no great factor, since then a person drinks more. It is also affected by the amount of fluid lost in other ways, particularly by diarrhœa and by vomiting.

Exudates (pleural or ascitic), œdema, and other abnormal accumulations of fluid in the body are excreted through the urine. This explains the polyuria in nephritis as the œdema disappears. It is beautifully seen if the person be put on constant fluid and the urine carefully measured; yet here also the excretion is not immediate, and may be distributed over so long a time that the demonstration fails.

The relative amount of urine voided during the *day and night* has not received the attention which it deserves. Quincke was first to call attention to this point. He and his students found that in liver, kidney, and heart diseases producing œdema the urine voided per hour during the night is greater in amount and contained more solids than during the day, a condition sometimes called n y c t u r i a. Normally the reverse is true, the kidneys seem to sleep with the rest of the body, and the amount per hour during the day is to the amount voided per hour during sleep as 100:50 to 60 or perhaps 80 to 90. The reverse is true in cases of cardiac or arterial disease and in nephritis, in which cases it would seem as if the kidney during the sleeping hours improved its opportunity to eliminate that which it could not during the day. In a well-marked case of nephritis, D:N::100:200, but in one case which we followed the ratio was even 100:544.[1] This does not depend, we are convinced, upon the mere position of the patient and the circulatory changes dependent upon this. This has some diagnostic importance to differentiate those cases of functional (*e.g.*, hysterical) from organic disturbances. The disturbed ratio is particularly marked in case the output be increased, as in diabetes, or by diuretics or by exercise during the day. It is not found in heart disease providing the compensation be good. Cardiac insufficiency seems the underlying cause in all cases.[2]

By POLYURIA is meant an increased output of urine, 3000 cc. being

[1] Johns Hopkins Hosp. Rep., vol. x. p. 323.
[2] See Laspeyres, Deut. Arch. f. klin. Med., August 16, 1900.

roughly considered as the upper physiological limit. If the output be below 800 cc. the term o l i g u r i a is used. The observation of one day is never sufficient; the increase or the diminution must extend over several consecutive days. These limits are very elastic, the controlling factor being the amount of fluids ingested, and the question always arising, is the polydipsia primary or secondary to the polyuria? For instance, in the cases of typhoid fever without any apparent renal disturbance Dr. Cole has been in the habit this year of increasing the diuresis as much as possible in hopes of increasing the elimination of the toxines, and outputs of from 6 to 14 litres a day were not rare. Here the polyuria was secondary to the consumption of larger amounts of water than the patients desired. In other cases also of typhoid fever after convalescence the output of urine is increased perhaps from the elimination of certain solids, and the increased intake of fluids is secondary to the tissue-thirst intensified by the depletion of water.

Pathological factors influencing the amount of urine are:

(1) The condition of the renal parenchyma; a bilateral diffuse lesion is usually necessary. The general law is that the more acute the nephritis the less the amount of urine, the more chronic the nephritis the greater the amount of urine excreted. In acute nephritis there may at first be anuria, or 50 to 100 cc. only, in a subacute nephritis about a normal amount, while in a chronic interstitial from 6 to even 12 litres in twenty-four hours. In the chronic cases the reason for the polyuria is uncertain. It cannot be blood-pressure alone.

(2) The velocity of the blood current through the kidney is of particular importance, the general law being that the amount of urine varies directly as the rapidity of blood-flow, not blood-pressure alone; that is, as the amount of blood passing through the kidney in a unit of time. Hence all cases of chronic passive congestion of the renal circulation due to whatever cause have a diminished output, and drugs which improve this circulation are called "diuretics." This is important in diagnosis and in prognosis.

(3) Disturbed metabolism. The output of urine depends much on the quality and the quantity of the substances excreted. The best illustration of this is diabetes mellitus, in which disease, because of the sugar elimination, even 25 litres of urine may be voided, and when, by modifying the diet, the sugar is much diminished, the water output diminishes as well. Similar may be the explanation of the so-called "epicritical polyuria." Some cases of typhoid fever, for instance when convalescence begins, void from 4 to 6 litres of urine per day; and in almost any disease causing diminished output, as the case improves the urine is much increased. This is beautifully seen in cases of nephritis, especially the chronic parenchymatous. The increased

water output following fevers may, as here, be due to the elimination of known or unknown bodies which were retained during the fever. This increased flow is a sign of favorable prognosis.

(4) Psychical disturbances and various nervous storms may be followed by polyuria; angina pectoris, hysteria, and after epileptic convulsions. The cause is probably a vasomotor one. The so-called "paroxysmal polyuria" is probably such a functional disturbance. Another cause of periodic polyuria is the periodic hydronephrosis seen in movable kidney, etc.

(5) There are certain other cases of polyuria the cause of which is unknown. The best illustration is diabetes insipidus, in which the output may be as high as twelve or more litres in a day. Meyer,[3] whose paper is a very interesting one, considers that the trouble in this disease is the inability on the part of the kidney to secrete a urine of normal concentration; hence the person must excrete large amounts of water to excrete the normal amount of solids.

In certain diseases the sequence is perhaps the following: The renal cells eliminate a greater percentage of the water of the blood than normal, due either to diseased condition of these cells, to circulatory disturbances, or to the increased output of some solid. This concentration of the plasma leads to a somatic thirst, hence the ingestion of an increased amount of fluid which is at once excreted. On the other hand, in the case of acute nephritis an oliguria may be due to the functional insufficiency of the cells to excrete water and salts. In other cases, *e.g.*, subacute nephritis, there is certainly retention of salts, and the water is retained as a result. When these salts are eliminated the water is eliminated with them. It is possible that in other cases the increased amount of water voided is the expression of an increased output of some unknown body.

It is often of interest to note what proportion of water intake is excreted through the kidneys. For normal persons 60 to 70 per cent. may be considered the usual limits, although the factors influencing it are many. If the water consumed be very much increased, the bulk appears in the urine and the percentage rises to even 96 per cent. (a case of typhoid fever, with ingestion of 6772 cc. of fluid). In two cases of chronic interstitial nephritis the relative output through the kidneys was high even when this output was small; one case with an intake of 1960 cc., 85 per cent.; the next day, of the 2400 cc. consumed, 86 per cent. was excreted by the kidneys. In another case, of 1370 cc., 85 per cent., and of 1790 cc. 83 per cent. were thus eliminated. In chronic parenchymatous nephritis, with the patient in almost stable condition and receiving exactly the same amount of fluid each day for 26 days (6200 cc. total), the average output was 66 per cent. With ascites and other signs of renal insufficiency it will drop to 40 per cent. or lower, even in anuria to 0. The following figures from a recent case of eclampsia in the obstetrical ward will illustrate this well. The patient was not urged to drink much. On the first day after the convulsions, of 8350 cc. of water drunk, the kidneys excreted 20 per cent.; the next day, of 10,535 cc., 80 per cent.; on the fourth day, of 9400 cc., 93 per cent.; and on the fifth the 7390 cc. of urine exceeded the intake of 7100 cc. During this time there was also some diarrhœa.

Careful observations were made on but few cases, hence the above figures are merely suggestive.

ANURIA may be due to a variety of causes, which may be grouped as obstructive, reflex, renal, and prerenal. It may be simply a nervous

[3] Deut. Arch. f. klin. Med., 1905, Bd. lxxxii.

symptom, seen in hysteria, in which case it is followed by a polyuria. It may be due to trauma, to the occlusion of the urinary passages, as, for instance, by stones, or a stone on one side and reflex anuria on the other, or to the reflex influence of nephrectomy on one side; or to the condition of the renal epithelium as in acute nephritis, tuberculosis, cystic disease, etc. There are certain other so-called "pre-renal" causes, —certain cases of fevers, as scarlet fever; poisons, as phosphorus, lead, turpentine, ether, chloroform; or collapse, and usually toward death, but not always.[4] In cholera the anuria has been attributed to the inspissation of the blood. In Moxon's case the anuria of fourteen days recovered after passage of the stone, and one case of nineteen days then recovery is recorded (Adams). Polk's case, whose only kidney was removed, lived eleven days.

Specific Gravity.—By specific gravity of the urine is meant its weight compared with that of an equal volume of water. The latter is usually expressed as 1000. This may be determined accurately by weighing in a pycnometer, but clinically it is determined by a form of aërometer called a urometer. These spindles are usually graduated from 1000 to 1050. It were better that two be used, graduated, the one from 1000 to 1020, the other from 1020 to 1040. As a result of our experience, we recommend that the practitioner, when he buys one, gets a good one. Some instruments on the market are inaccurate, especially those designed for use with a small volume of urine. The specific gravity of the urine can be put to good use, and the best instrument is none too good. The urine glass used should be a cylinder with parallel sides, wide base, and a good spout. The fluted side of the Squibb's model is an advantage. Such a glass is filled about four-fifths full of urine in such a way as to avoid foam. If present, this may be removed with a piece of filter paper. The bobbin is then dropped in, the observer assures himself that it neither rests upon the bottom nor touches the side, in which latter case it will certainly register from 1 to 2 points higher than it should. The reading is made with the eye on a level of the base of the meniscus. Two or three readings should be made, the bobbin being pushed down and allowed to come to rest each time. There is one point of considerable importance, and that is that these instruments are standardized at a certain temperature, usually at 15° C., and a difference in temperature of 3° means a difference of 1 in the fourth place of the specific gravity reading. Hence a urine which at 15° C. has a specific gravity of 1012, at 18° C. will read 1011. This is, of course, usually of slight importance with urines of ordinary concentration, yet we suspect that it explains the phenomenally low specific gravity in certain cases of diabetes insipidus and chronic interstitial

[4] See, also, Bevan, Am. Surg., April, 1903.

nephritis. This correction is, of course, indispensable if the specific gravity is to be used in quantitative work, as, for instance, the estimation of the total solids or the amount of sugar or of albumin. It is only just to say that for the latter we think the aërometrical method at its best is hardly accurate enough, and the urine should if possible be weighed on a good chemical balance. Again, an instrument suited for salt solutions is not always accurate in a sugar or albumin solution.

The twenty-four hours' specimen should be examined; this only has very much value, for the various portions during the day and night may vary from 1002 to 1040, depending on the food, the fluid, the lungs, the skin, etc. It may be very high after severe exercise with sweating, after transudate formation, etc. Two cases recently were refused on first examination by life insurance companies because they happened to have eaten some food just before examination which for them was a diuretic, hence an abnormally low specific gravity was found—in one case as low as 1003.

The normal specific gravity is from 1015 to 1020. In the newborn, 1005 to 1007.

In case there is too small an amount to fill the tube, it may be diluted to a known volume, the formula for the correction being: Sp. gr. $= 1000 + ab$, in which "b" = dilution, and "a" = the last two figures of the specific gravity found. For instance, if the urine was diluted with just twice its volume of water, and if the reading of the diluted urine was 1006, Sp. gr. $= 1000 + 3 \times 6 = 1018$.

In some cases it is not the specific gravity of the twenty-four-hours' specimen which is desired. For instance, in the diagnosis of an early chronic diffuse nephritis the constantly low specific gravity of the morning urine is of value. In general, however, if one has not a total mixed specimen, the specific gravity would better not be determined. This figure is in some clinics put on the temperature chart together with the amount of urine, the reason being that neither figure means much without the other.

The specific gravity depends chiefly on the amounts of water, urea, and sodium chloride present. The water will depend on the same factors already discussed under amount. The urea explains to a certain degree the high specific gravity in fevers. The amount of salts is increased by foods, by the medicines taken, and by the absorption of transudates. While in general the specific gravity will vary inversely as the amount, this is not strictly true, since the output of solids is always increased by an increased output of fluids. A noted exception is that of diabetes mellitus, in which with increased amount is also an increased specific gravity, from 1025 to 1040; and in nephritis with renal insufficiency, in which case with oliguria the solid

output is diminished. In nephritis a low specific gravity is rather suggestive of an impending uræmia. It is also seen, however, in cases of malnutrition in which the metabolic processes are at low ebb, as for instance in one of Chabrié, a girl of twenty years of age, whose output on one day was 750 cc. with a specific gravity of 1008. In diabetes insipidus the specific gravity is very low. We doubt, however, some very low figures given, suspecting that the temperature has something to do with one or two points. With normal amounts of urine there is a rather high specific gravity after operations, in rheumatism, and in rickets. Ether anæsthesia diminishes the specific gravity, the amount remaining about normal (Brown).

To determine the amount of solids in the urine an approximate estimation may be made by the use of Häser's coefficient, 2.33. The last two figures of the specific gravity multiplied by this empirical coefficient will give a clinically accurate estimation of the number of grammes per litre of solids excreted.

It is surprising how much use may be made of the simple determination of the specific gravity in quantitative work; *e.g.*, sugar, albumin, etc. To have much meaning, however, the variation should be present on several successive days, since the physiological variations are considerable.

Others disagree concerning this coefficient. Neubauer gives 2.328; Donze[5] states that the coefficient should be slightly lower for dilute than for more concentrated urines, varying from 1.850 to 2.440, with an average of 2.210.

Color.—The color of normal urine is usually a shade of yellow. This varies with the dilution of the urine, and hence directly with the specific gravity, being pale in the dilute and dark in the scanty urine. Exceptions to this are diabetes mellitus, in which case it is very pale and yet the urine large in amount and of a high specific gravity, a point which will sometimes suggest the condition; in anæmia, especially chlorosis, in which case the urine is pale from lack of pigment, since the hæmoglobin is the chief source of the urinary pigments, but not in those anæmias in which there is rapid destruction of the reds, as in pernicious anæmia; here the urine is highly colored. In general, acid urine is more highly colored than alkaline. In uræmia it may be so pale that formerly it was thought the pigment retained because of renal insufficiency was the toxin causing the condition. In certain grave infections destroying the bile-producing function of the liver the urine is said to be without pigment. A febrile urine is dark, since concentrated, and also from the presence of uroërythrin and other pigments which manifest themselves especially after it is exposed to the air. The contrast in color between

[5] Compt.-rend. Soc. de Biol., 1903, 155, 537.

the day and night urines is often striking, the day urine being of a golden-yellow, the night of a pale green color. This is due partly to the amount of pigment excreted, partly to the effect of sunlight on the specimen collected during the day.

A color scale is very convenient to use (such may be found in Purdy's " Analysis of the Urine," and Neubauer and Vogel's chart is published large for the urine examination-room by Kreidel, of Wiesbaden), since a variety of terms is used in describing the same color (yellow, light yellow, amber, straw, etc.) With this scale should be compared urine in vessels of a certain depth, and against a white background.

The pigments normally present in the urine are:

UROCHROME which is the one chiefly responsible for the normal yellow color. This is the predominant pigment of the urine, giving colors varying from yellow, orange, to brown, according to the amount present. It has not yet been isolated, its empirical formula is not yet known, and there may be several pigments included under this name. It has no absorption spectrum, no fluorescence. There is evidence that it is derived from urobilin.

HÆMATOPORPHYRIN is in small amounts normal in the urine (see pages 92 and 236).

UROËRYTHRIN is normal in certain cases. It explains the salmon-red color of the urate sediments. It is increased by a rich meat diet, profuse sweating, alcoholic drinks, violent exercise, and by certain digestive disturbances; also in fever, circulatory disturbances of the liver, and rheumatism. It may be demonstrated by shaking the urine out gently with amyl alcohol, which will take an orange color and give the characteristic spectrum. This pigment bleaches in a characteristic manner on exposure to light. With concentrated sulphuric acid its solutions are carmine-red, which on the addition of an alkali changes from purple, to blue, to green.

UROBILIN is normally present, from 30 to 120 mg. per day. The pigment is itself not at first present, but a chromogen, urobilinogen, which on exposure to sunlight gives urobilin. Whether there are several urobilins, as some think, or not, is a matter of considerable dispute. It is so hard to isolate this body without a certain amount of decomposition, and so hard to exclude impurities, that the question is still unsettled. Its origin also is a matter of some dispute. One thing is quite definite,—that a certain amount is formed in the intestine as the result of the reducing action of certain bacteria on the bile pigments, *enterogenous formation*. It is the same as stercobilin, which certainly is derived from bile pigments in this way. It is not the same as hydrobilirubin. In favor of this origin is its absence in the urine of the new-born, after complete bile obstruction, and its

increase in intestinal decomposition. It may, however, arise elsewhere, since it is increased in cases with blood extravasation into the tissues (*histogenous*), and in disease with increased blood destruction, as also after blood poisons such as antifebrin and antipyrin (*hæmatogenous*). It is increased after a biliary obstruction is relieved. It is increased in fevers, chronic passive congestion, lead-poisoning, atrophic cirrhosis of the liver, and especially in conditions accompanied by urobilin jaundice, which list includes many of the above, and in any disease producing jaundice excepting the catarrhal. Another view is that of Gilbert and Herscher and others,[6] who consider that the kidney reduces the bilirubin to urobilin, since the latter is a more easily diffusible pigment. They point out that even when abundant in the urine none can be demonstrated in the blood unless much bile also be present; that in a light case of cholæmia there will be no bile in the urine, only urobilin; in the moderate cases both may be present, while in the severe cases in which the exhausted kidneys seem to give up their task of transforming this pigment only bile is present. Meinel[7] finds in a very few cases urobilin formed in the stomach in cases of hyperacidity, although this may be of enterogenous origin (Braunstein). The French writers especially emphasize urobilin as an index of the condition of the liver, its presence indicating functional hepatic insufficiency, and consider an abundant increase a faithful sign of traumatic lesions of the liver.[8] And lastly is the view that if there be no liver disease and the kidneys normal, urobilinuria always means autohæmolysis.[9]

Urobilin does not give the Gmelin test. It does give a test similar to the biuret. If to the urine made strongly alkaline with ammonia and filtered be added a 1 per cent. alcoholic solution of zinc chloride, there will be seen a beautiful green fluorescence, and the absorption bands of alkaline urobilin may be found. This spectrum is characteristic. That of acid urobilin may be determined in a urine directly if a few drops of a mineral acid be added, but it is better to shake out with amyl alcohol and examine the extract. Or to the urine may be added an equal amount of 10 per cent. ZnAc in absolute alcohol, and the mixture filtered.[10] This test is given even in the presence of considerable bilirubin. The fluorescence is best seen with a convex lens, which gives a luminous green circle.

For quantitative work, Hoppe-Seyler's method is recommended. One hundred cc. of urine are acidulated with sulphuric acid, saturated with ammonium sulphate,

[6] Compt.-rend. Soc. de Biol., 54, p. 795.
[7] Centralbl. f. inn. Med., 1903, vol. xxiv. p. 321.
[8] See also Rolleston's case of urobilin jaundice following trional in a case with nutmeg liver, Brit. Med. Jour., 1897, i. p. 719.
[9] Erben, Prag. med. Wochenschr., 1904.
[10] Schlesinger, Deutsch. med. Wochenschr., 1903, No. 32, p. 561.

and allowed to stand for some time; then filtered, and the precipitate washed with saturated ammonium sulphate. The precipitate is then pressed out between blotting-paper, extracted with equal parts of alcohol and chloroform repeatedly. The extract is then filtered into a separating funnel, and to the filtrate is added two volumes of water and then chloroform until the chloroform settles out well in a clear layer. The chloroform solution is evaporated on a water-bath and the residue dried at 100° C. It is then extracted with ether, the ether extract filtered off, the residue dissolved on the paper in alcohol, again brought into the weighed beaker, evaporated, dried, and weighed.

The spectrophotometric method of Friedrich Müller may be used.

Among other chromogens in the urine are indoxyl-sulphuric acid, indoxyl-glycuronic acid, perhaps skatoxyl-sulphuric and skatoxyl-glycuronic acid. Pathologically, among the pigments present may be hæmoglobin, methæmoglobin, hæmatin, bile pigments, melanin, and others; from drugs, chrysophanic acid *et al.*; from the foods, the pigments of various berries, cherries, etc.

Blood.—The color of the urine when blood is present depends upon the amount and form of the blood pigment, hæmoglobin giving in general a reddish tint, and methæmoglobin a brownish one. The urine may therefore grossly be of a reddish-brown, brown, almost black, or greenish-black, as in the black-water fever of hæmoglobinuria. When little is present it often has a characteristic smoky tint of methæmoglobin, which should always suggest blood. The urine is cloudy because of the large number of corpuscles and other organized elements of sediment usually present. In the heavy sediment are masses of amorphous hæmoglobin.

Hæmatoporphyrin.—This is present in large amounts after the long use of trional, sulphonal, tetronal; also in cases of typhoid fever and other diseases. Thick layers of the urine have a dark or a blackish color; thin layers a yellowish-red or violet. The black color Garrod thinks due only partly to this pigment, and more to an unstable purple one.

Bile.—When the patient is jaundiced the urine usually contains bile, but in cases of very mild jaundice urobilin alone may be present. If bilirubin and biliverdin are present, the color of the urine will often be dark yellow, brown, green, or even greenish-black or quite black if considerable biliverdin is present together with bilirubin and other bile pigments, especially in long-standing cases (Garrod). If it stands a long time in the cold there may be a sediment of bilirubin in needle crystals, especially if the urine is very acid. It is often possible to detect the presence of bile in small amounts by producing a foam by shaking the urine. This foam, always white in other urines no matter how dark they may be, is stained yellow by bile; it is also yellow in case very much urobilin is present.

Melanin.—This rare pigment is present in cases of melanotic tumors which have invaded the viscera. Garrod finds the amount

of melanin to depend upon the involvement of the liver especially. The urine is usually of a perfectly normal color when voided, since the pigment is present as a chromogen, melanogen, which later splits giving melanin. But it may be black when voided. This transformation may be hastened by the addition of nitric acid or other oxidizing bodies to the urine. It begins at the top and extends downward forming sometimes very strikingly a sharply defined layer above the colorless urine. Ferric chloride causes immediate blackening and a gray precipitate soluble in excess. Unless this reaction is positive, melanin cannot be assumed present, as it is the most delicate and reliable test (v. Jaksch.)

Homogentisinic acid, the chief coloring body of alkaptonuria, gives the urine a brownish-black color and a syrupy consistency after standing or after the addition of an alkali (see page 197).

The urine is sometimes very dark in peritonitis, gangrene, and other conditions with the formation of aromatic products of decomposition, the ethereal sulphates of indoxyl, etc. In these cases the blue color sometimes seen is not indigo, but a higher oxidation product of indol. Such urines blacken on warming with nitric acid, not when cold, do not blacken with ferric chloride, and do not reduce copper solutions. In one striking case in our wards the fresh urine of a woman who had been markedly constipated was of a very dark greenish-black color, but after the bowels had moved well the next voiding was of practically normal color. Some indican was present, but not nearly enough to explain the color.

In some cases the urine is very dark on voiding; in others after long standing. This may be due to *pyrocatechin*, $C_6H_4(OH)_2$(1, 2), which in watery alkaline solution is oxidized by the air and becomes a greenish-brown and finally a black color. The urine containing it becomes therefore dark, reduces alkaline copper sulphate in warm solution, but not bismuth. Another view (Baumann) is that pyrocatechin is derived from the vegetables of the food.

To isolate, the urine is concentrated, filtered, a little sulphuric acid added, and then boiled to drive off the phenol. It is then shaken out repeatedly with ether; the ether is distilled off, the residue neutralized with barium carbonate, and shaken out again with ether. The ether is then evaporated off and the pyrocatechin allowed to crystallize out.

Hydrochinon, $C_6H_4(OH)_2$(1, 4), occurs after the use of phenol. Its decomposition product gives a dark color to the urine and reduces copper easily.

Urine containing the alkapton bodies and indican is clear on voiding, but soon becomes dark. In the latter case the blue of the indigo may not be pronounced, since modified by the yellow of the urine, although the scum may be blue. Sahli mentions the case of a boy

in which the urine when voided was of a green-grass color due to the combination of the blue of the indigo with the yellow of the urine.

Ochronosis, a disease with blackening of the cartilages, has been reported as a rare condition in which the urine blackens on standing. In Osler's cases alkaptonuria was also surely present, but in other cases it was said the urinary pigment was not that of alkaptonuria.

In certain rare cases the urine on voiding is black, or colorless soon turning black. The pigment causing this has not been determined, but all the above mentioned causes are excluded.

Garrod [11] classifies the *black urines* as, those due to long-standing jaundice; certain cases of hæmaturia, hæmoglobinuria; melanotic sarcoma; alkaptonuria, ochronosis; great abundance of indoxyl-sulphate; certain cases of tuberculosis after standing for some time, a month even (the cause not known); perhaps phenol derivatives, certain drugs as phenol; and rare cases due to an unknown pigment. Those truly black are only melaturia, and alkaptonuria on standing.

In *chyluria* the urine is of milky appearance.

Colors due to Medicines.—The list of medicines which may affect the color of the urine is too long to tabulate. In general, it may be said that in case the urine presents any unusual color, inquiry should always be made concerning the previous medication. Among these drugs particularly are carbolic acid, whether applied internally or externally, tar preparations, resorcin, naphthol, salol, and many aromatic bodies. The color in these cases often appears only after long standing, and especially when the urine is alkaline, and when hydrochinon and pyrocatechin are formed. *Methylene blue*, even in small amounts, 0.1 gm., will color the urine for several days. Hence the result was startling in cases of malaria treated with large doses of this drug. In one hour after the dose the urine has a greenish color, later a deeper green, then a blue, which may last three to four days. The color may be intermittent, present only in the first morning voiding. It may be intensified or produced by boiling the acid urine, adding acetic acid if necessary, since the pigment is partly reduced in the body to a colorless form. Weber [12] thinks methylene blue explains practically all the blue and green urines, and doubts cases ascribed to indigo blue. He emphasizes the common use of this dye to color candies and food-stuffs.

Some colors are of clinical importance only in case of a drug applied externally and hence in uncontrollable doses. What the factor is which changes the color of the urine cannot always be determined; for instance, after a small dose of salol the urine may be of a very

[11] The Practitioner, 1904, vol. lxxii. p. 383.
[12] Lancet, September 21, 1901.

dark color, while after much larger doses there will be no change. Whether this is due to the acidity or to the time of exposure to the air cannot be said.

After drugs containing *chrysophanic acid*, as, for instance, chrysarobin, rhubarb, santonin, senna, and others, the urine is of a yellow tint when acid and red when alkaline. The pigment of many vegetables will change the color of the urine. Among these may be mentioned turnips, whortleberries, blackberries, and others.

Odor.—The odor of the normal fresh urine is not unpleasant. The so-called urinary odor is due to the ammoniacal decomposition by the bacteria. In a decomposing albuminous urine the odor is especially disagreeable, and a diagnosis of albuminuria may be made from that alone. There is said to be an intolerable odor in cases of cancer of the bladder and deep inflammatory disease of the urinary tract. Chabrié believes in a characteristic odor in certain cases of abnormal metabolism with incomplete combustion, such as is present in diabetics and oxalurics. We may even suppose that he thinks that one of the great masters of French medicine could diagnose insanity from the odor of the urine alone. There is said to be a special odor in chyluria and even in slight hæmaturia. Other cases have a remarkable absence of odor. It should always be remembered, however, that the bottle in which the patient brings the specimen may explain the odor. We have noticed a strong odor of H_2S in certain nephritics, even when the urine was quite fresh.

Certain substances are excreted as such in the urine. Among such are valerian, asafetida, coffee, and various foods. Others build odorous bodies. Among these are the balsams, copaiba, cubebs, etc. After the administration of turpentine the odor of the urine is that of violets. After eating asparagus there is a characteristic odor attributed to methyl-mercaptan.

General Appearance.—When fresh the urine is clear. If there is then any distinct cloudiness, it is due to an abundant organized sediment or to a precipitation of phosphate seen in the so-called phosphaturia and in ammoniacal cystitis. Very soon a faint nubecula appears in the upper layers of a clear urine, which consists of mucous strands enclosing a few cells. After standing, the urine will become cloudy, either from a urate sediment, which before settling may give a uniform milky appearance, or particularly during the summer months to the rapid growth of bacteria and the precipitation of the phosphates in the alkaline urine.

Reaction.—Concerning the reaction of the urine there has been much work done in regard to the value of which much difference of opinion exists. All admit that its determination would be valuable could satisfactory methods be found.

Until recently by "degree of acidity" was understood the amount of hydrogen which could be replaced by the metal of an alkaline solution (NaOH), regardless whether these hydrogen ions were already dissociated or could be substituted by the alkali. Now is meant the absolute number of dissociated H-ions per one litre of urine. The latter contribution of the physical chemists is interesting, indeed, but of little value to the clinician. Judged by this, urine is only about thirty times as acid as distilled water, and only about one ten-thousandth as acid as titration would indicate, and the difficulties of its determination rule this out from clinical methods. The titration method alone is possible for general use, and the question arises if its results are of any real value or simply of an empirical arbitrary value. Höber[13] claims, as the result of parallel estimations, that these two "acidities" vary sometimes, often perhaps, in a very independent manner, hence variations in each would have different values, and neither method would be able to replace the other. The question, therefore, is, Does the titration method give results valuable enough to repay the time, or are the results worse than useless since misleading? The difficulties are that the acidity of the urine in the common sense of the term depends upon a considerable number of chemical substances, for the most part acid salts, and hence the question of color indicator is a very serious one, since the points indicated by the various ones as the neutral point differ and none is by any means the theoretical one. Phenolphthalein is the one usually used. This has as practical advantages the sharpness of its end reaction and the fact that of the indicators it is itself the weakest acid. But it is a poor indicator in the presence of ammonium salts, perhaps the worst. Whatever results are obtained with it must be given not an absolute but an empirical value. Yet the opinion of those working in this line is that the results with it are comparable.

The reaction of the twenty-four-hour amount of well-preserved urine is, in the case of man, always faintly acid to litmus, a degree corresponding to about 1.15 to 2.3 grammes of HCl for twenty-four hours. This acidity depends chiefly upon the diet, and is greater the more the proteid oxidized. The urine of herbivorous animals is alkaline, since the organic acids of their food are oxidized to alkaline carbonates, yet if starved, acid, since then their tissue proteid is their diet. A man on a vegetable diet will have a less acid, or amphoteric urine perhaps, from this increased ingestion of alkali-forming foods. In no case is there free acid in the urine, the acidity being due to acid salts, and particularly diacid sodium phosphate. There are many other acids produced in the oxidation of proteids themselves neutral. Among these are sulphuric, phosphoric, uric, hippuric, oxalic, and the

[13] Hofmeister's Beitr., 1903, vol. iii. p. 525.

oxyaromatic acids. Just what part these play, however, cannot be decided, but certainly uric acid is no factor, since its solution is neutral to litmus.

A constant acidity has been found only after and during a period of starvation.

Variations in the reaction are due to the diet, as mentioned above. The acidity is highest in the morning before breakfast and lower a few hours after each meal, and especially in the forenoon, due to the secretion of hydrochloric acid of the gastric juice. For a short time, two to four hours, after a meal the urine may, indeed, be alkaline when freshly voided and turbid with sediment of the phosphates of the alkaline earths, a condition known as "phosphaturia." Normally this diminished acidity or even alkalinity of the "alkaline tide" diminishes after a meal during the time the hydrochloric acid is reabsorbed.

PHOSPHATURIA is the term formerly given to a symptom-complex with a heavy precipitate of the earthy phosphates in the freshly voided urine, yet without the formed elements which would indicate a lesion of the tract, and supposed to be due to an increased output of this acid. Chemically, however, there is no increase, and what is more important, often a decrease, and the name "alkalinuria" is more suitable. Such cases are due: first, to a diet which raises the alkalinity of the blood, as a vegetable one; in gastric diseases, with considerable loss of hydrochloric acid to the body through hypersecretion with motor insufficiency and vomiting or lavage, perhaps diarrhœa also; and especially as a symptom of neurasthenia (Peyer) without any of the above-mentioned causes. In such a case during the periods of neurasthenia has been found a diminution in the phosphoric acid to about half, but an increased calcium output. The nitrogen was also decreased. It seems to be the excess of calcium relative to the phosphoric acid which leads to the precipitation. In Soetbeer and Krieger's case the phosphoric acid was practically normal, the calcium increased even to 0.7 gm. a day (normal 0.2) and Ca : P_2O_5 :: 1 : 1.5 to 2 (normally 1 : 12). In certain cases[14] there seem to be during the period of phosphaturia symptoms referable to this abnormal metabolism, and relief with its disappearance, but perhaps connected more with the change of calcium metabolism than of phosphoric acid. In one case the calcium was increased over three times, perhaps the result of catarrh of the colon. It occurs also in persons after sexual excesses, and in the depression following psychical exaltation, in which cases the cause is not known, but a nervous control suspected. Freudenberg[15] carries this to an extreme, separating phosphaturia, latent phospha-

[14] Soetbeer and Krieger, Deut. Arch. f. klin. Med., 1902, vol. lxxii. p. 553; Patek, M. J., vol. xxx.

[15] Deutsch. med. Wochenschr., September 17, 1903.

turia (in which the precipitate appears on heating the fresh urine), and ammonuria (tested by moist litmus over the mouth of a tube of heated urine), three grades, he thinks, of the same abnormality, and which he found in sexual neurasthenics especially, not in patients with hysteria. It is often found among mental cases (Heinicke). Some few cases with general symptoms have really increased phosphoric acid as their only objective sign; later, perhaps, polyuria or glycosuria. Senator suggests that some cases of diabetes insipidus with rather high specific gravity may belong here.

The reaction of the urine can be much modified, even made alkaline, by drugs, particularly by alkaline salts in large doses. Milk of lime will give an alkaline urine due to the presence of ammonium carbamate (Abel). While a transudate is quickly absorbed, the urine may become alkaline; also after hemorrhage of the intestine, in which case the blood salts are absorbed. It is alkaline in certain cases of pneumonia, typhoid fever, and diseases of the central nervous system. We have noted a marked alkalinity in certain cases of nephritis, particularly of the severe chronic parenchymatous form with much œdema, which renders the examination of casts difficult. The urine is also alkaline when there are alkaline secretions and exudates of the urinary tract, as in cases of cystitis or urethritis; and lastly in alkaline fermentation in the bladder.

The alkalinity is, of course, usually due to the changes occurring after the urine is voided. Bacteria begin at once to break the urea up into ammonium carbamate and carbonate. For this some suppose a special ferment.

It is of importance to determine whether the alkalinity be due to a fixed alkali or to ammonia. If the latter, it is always the result of bacterial fermentation. This may be determined by wetting red litmus paper in the urine and then drying it; if ammonia, the red color will return: or moist litmus paper hung in the mouth of the bottle will, if much ammonia be present, turn blue. But even normal urine contains a certain amount of ammonia, and hence the paper if left long enough will usually blue somewhat.

The acidity of the urine can with difficulty be increased, and not beyond a certain point. This occurs with increased proteid metabolism. Cases of hyperacidity, even two to five times normal (phenolphthalein as indicator), and accompanied by symptoms of cystitis, pain especially in the trigonal region, but without demonstrable lesions or assignable cause, are reported by Brown [16] in cases of girls and young women of distinctly neurotic temperament. He suggests that it is a neurosis of urinary secretion. The urine is very acid in diabetes mellitus if it contains considerable oxybutyric and diacetic acids. The

[16] Phila. Med. Jour., March 2, 1901

question of the reaction in the so-called "uric acid diathesis" is not yet decided. The reason that it is so difficult to increase the acidity in the case of man is that the body will protect itself against an acid intoxication by an increased excretion of ammonia, thus protecting its native mineral alkaline store from depletion. This ability is present in the herbivora to a much less extent, and hence they are more easily poisoned by acids than is man.

The effect of muscular work on the urine reaction is still unsettled. As the urine decomposes, in some cases after six to twelve hours of standing, it becomes more acid, the so-called "acid fermentation." The reason of this is uncertain. It is inconstant and is always soon succeeded by an alkaline decomposition. Hammarsten considers it due to the reaction between the biurates and MH_2PO_4.

Determination of the Total Acidity of the Urine.—Naegeli[17] advised to add the N/10NaOH directly to 10 cc. of urine, phenolphthalein used as indicator. The error is at least 4 to 8 per cent.

Folin[18] uses potassium oxalate in excess to rule out the error from ammonium salts and calcium phosphate.

Twenty-five cubic centimetres of urine are measured by a pipette into a 200-cc. Erlenmeyer flask, one or two drops of 0.5 per cent. phenolphthalein solution added, and 15 to 20 gms. of potassium oxalate. The flask is shaken well for one minute, then at once titrated with N/10NaOH.

(For the Freund method, see page 129.)

THE NITROGENOUS BODIES

The Nitrogen Output.—The total nitrogen of the urine is the best index of proteid metabolism. It is fortunate that for this, our stand-by in metabolism work, we have a satisfactory method of determination. The same cannot be said, however, of the several nitrogenous bodies.

In the normal adult on an average diet the total nitrogen output is from 10 to 16 gms. per day.

According to Hammarsten this is distributed about as follows:

	Adult. Per Cent.	Infant. Per Cent.
Urea	84 to 91	73 to 76
NH_3	2 to 5	7.8 to 9.6
Uric acid	1 to 3	3 to 8.5
Extractives	7 to 12	7.3 to 14.7

The sum of the nitrogens of urea and ammonia bears a very constant relation to total N (91 to 93 per cent.), a much more constant one than does either alone.[19]

[17] Zeitschr. f. physiol. Chem., 1900, xxx. 313.
[18] Am. Jour. Physiol., 1903, ix. 265.
[19] Folin., Am. Jour. Insan., 1905.

In general the total nitrogen is increased as a result of increased proteid metabolism, a heavy proteid meal, or anything increasing body proteid catabolism. Less is excreted on a diet rich in carbohydrates than even while fasting, since in the latter case the body lives on its tissue proteid. The output reaches its maximum a few hours after a heavy proteid meal. The evidence given that exercise increases the output is in part that the amount excreted during the day is to the amount excreted at night as 3 : 2. We hardly think that this alone is sufficient evidence, for it has been only too well shown by studies of the day and night urine that normally the kidneys can rest at night as well as the rest of the body. Hot baths increase the nitrogen output.

With increase of water excretion that of nitrogen also is increased. This latter point is important, for even when the diet is fairly constant, if by any reason the amount of urine be increased, the nitrogen also will rise. One explanation given is that the renal cells are always stored with a certain amount of nitrogenous waste which the water constantly removes; others say that the many tissue ferments, following the general law of ferments, act better in dilute solution.

Pathologically nitrogen is increased: in fever, owing not to the temperature *per se*, but more likely to the effect upon metabolism of the toxines causing the fever,—excepting acute nephritis causing dropsy and those with diarrhœa or with large exudates; in cachexia, in which cases there is a more rapid breaking down of tissue proteid; in diabetes, since even with a mixed diet such patients live on almost pure proteid from food and tissues; after various poisons, as arsenic, antimony, phosphorus, and other protoplasm poisons; and anything diminishing the oxygen intake, as prolonged dyspnœa, hemorrhage, carbon monoxide poisoning, etc. During the resolution of a pneumonic exudate its digestion and excretion can be well followed by the nitrogen of the urine, and the amount of lung cleared estimated. In a case of Müller's the excess of nitrogen output during the resolution was 28 gms., which represented 800 gms. of exudate. The continued large output in cases of delayed resolution would indicate a continuous process, a "chronic pneumonia," rather than a failure to resolve. Cook[20] followed a few such cases, in one of which, with one lung involved, the nitrogen output would represent the exudate of four solid lungs.

In much of the work on metabolism the urea, not nitrogen, has been followed, and by methods which determine really more the total nitrogen, as, for instance, Liebig's, or which are quite faulty, as Hüfner's.

By combining the findings of these two lines of work, to the above may be added that in general anything increasing proteid

[20] Johns Hopkins Hosp. Bull., December, 1902, p. 307.

catabolism increases the nitrogen. Again, anything increasing the water output will increase the nitrogen, as, for instance, in diabetes insipidus (in which disease 130 gms. of urea have been reported) and cases of chronic nephritis with polyuria. Again, the nitrogen is increased when exudates or transudates are absorbed.

Retention of nitrogen occurs in a person gaining weight, in myxœdema, in the convalescence of fevers. In one case of convalescent typhoid (Lüthje) the record was reached, in twenty-six days the person retaining 121.38 gms. of N, which would represent 758.6 gms. of albumin or 3568.6 gms. of muscle. The person gained 6490 gms. in weight. This retention of water and nitrogen is well seen in the last of pregnancy, and is followed by a diuresis and increased nitrogen output, which begins about the second day of the puerperium and lasts about two weeks.[21]

The amount considered normal, 10 to 16 gms. per day, is this high because we eat almost twice too much. Vegetarians can accustom themselves to a 5 to 6 gms. output and even then store up nitrogen.

The amount excreted is diminished physiologically by a poor diet, and especially by one rich in carbohydrate; when the water is reduced as a result of free sweating, etc.; in pregnancy; and by small doses of quinine. Pathologically, it may be diminished by a diminished absorption of the proteid in the intestine; or the oxidation in the body may be at low ebb, as in cases of very reduced vitality, and at the end of acute fevers. The diminution may result from the retention of bodies which should be excreted, as in cases of dropsy, exudate or transudate formation, also when there is a very small water output; or there may be an inability to excrete, as, for instance, in a severe nephritis in which the drop in urea may be one of the first serious symptoms heralding the onset of uræmia. This inability to excrete is also seen in mere functional disturbances of the kidneys; also before death due to any cause. In some cases it may be manifested by the urea frost, the urea crystallizing on the skin.

Estimation of Nitrogen.—The Kjeldahl method is quite uniformly used. Of this there are several modifications. That most commonly used is Gumming's. For all modifications it is necessary to have combustion flasks of Jena glass of about 250 to 300 cc. capacity, and an ordinary distilling apparatus with a good cooling jacket (see Fig. 19, C). To the urine, in amount varying from 5 to 20 cc. according to its concentration, are added 15 cc. of pure concentrated sulphuric acid, 10 gms. of potassium sulphate, and about 1 gm. of copper sulphate. This, supported on a sheet of asbestos gauze, is then boiled over a free flame in a hood with a good draft until the fluid is a clear blue. The worker should be careful, if it is necessary to wash down

[21] Slemons, Johns Hopkins Hosp. Rep., vol. xii. 1904.

the carbon from the sides of the glass by shaking the fluid, that he does not burn himself with this exceedingly hot acid. After the fluid is perfectly blue the heat should be continued for a few minutes or even half an hour, that the combustion may be perfect. Uric acid and other bodies are perfectly oxidized only after at least half an hour's further heating of the clear fluid. By this means practically all of the nitrogen has been converted into ammonia, and is hence

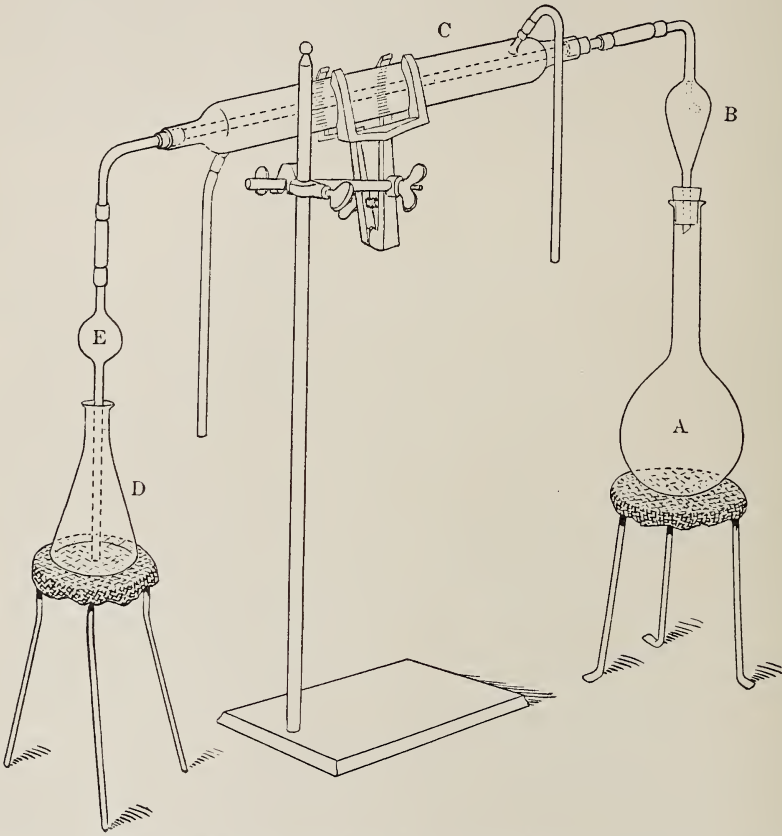


FIG. 19.—Distilling apparatus for nitrogen determination (Kjeldahl). A, Distillation flask; B, safety bulb; C, Liebig cooler; D, Erlenmeyer flask to receive distillate and containing the standard acid; E, safety bulb to prevent back-flow.

present as ammonium sulphate. The oxidation may be aided by adding a little $KMnO_4$. The fluid is allowed to cool perfectly, distilled water then added in excess, and the fluid poured into a distilling flask (see Fig. 19, A) of 1 litre capacity, with long neck and round bottom, and the combustion flask well washed into this flask, rinsing three or four times. Talcum powder or zinc granules may be added to prevent bumping. An amount of strong sodium hydroxide, specific

gravity 1230, found by previous experiments sufficient to more than neutralize the acid, is now added and the flask at once fitted to the Liebig cooler. The lower end of this cooler ends in a bent tube which vertically descends to the bottom of a small Erlenmeyer flask, D, of about 300 cc. capacity, in which have been put previously just 50 cc. of fourth-normal H_2SO_4. In the subsequent distillation, therefore, all the ammonia, which will be given off at once in the cold and the rest on boiling, bubbles through this acid and is thus caught. The distillation is continued until about 100 cc. of distillate have passed over, but the boiling should never be too vigorous, and the apparatus watched to be sure no acid spurts into the cooler; hence the vertical tube has a safety-bulb, B, to prevent this. The Erlenmeyer flask may then be lowered and the distillate tested with lacmoid paper, to make sure that the ammonia has entirely passed over. The acid clinging to the end of the tube is washed into the flask. This sulphuric acid is then titrated against fourth-normal NaOH, using cochineal, methyl orange or pure litmus as indicator. There can be no doubt that the pure litmus is the best if the necessary precautions are used. The most convenient is cochineal, which can be used in artificial light as well, and is sufficiently correct for ordinary work. (The cochineal bugs are ground finely and extracted with 50 per cent. alcohol. The filtered extract is used as indicator.) From the 50 cc. are subtracted the number of cubic centimetres of fourth-normal NaOH, and the difference indicates the amount of fourth-normal H_2SO_4 neutralized by ammonia. This value multiplied by 0.0035 gms. would equal in grammes the weight of nitrogen in the amount of urine used.

(NOTE.—This method does not indicate nitrates or nitro-compounds.)

For the relation between CARBON AND NITROGEN, see Richardson.[22]

Urea is the chief nitrogenous body of the urine and the one which until recently has attracted most attention. The methods employed to determine it (Liebig's, Hüfner's) have not given perfectly correct results, the first, in fact, giving a fair nitrogen determination, and the second, a fairly correct in some, a very incorrect result in other cases; hence among the figures reported in such mass many must be erroneous.

The amount of urea as an index of nitrogen metabolism has been used as a test of the digestion. In this case a meal containing an excess of nitrogen is given, for illustration, 500 gms. of meat, eight eggs, and 200 gms. of bread; during this and the following day at least 50 gms. of urea should be excreted.

The amount of urea excreted by a normal person on an average

[22] Am. Jour. Med. Sci., 1902, vol. cxxiv.

diet varies from 20 to 40 gms., more in the case of men than of women. On a poor diet it may be from 15 to 20 gms., while on a very rich diet figures as high as 100 gms. in twenty-four hours have been reported. In general it may be said that for a vigorous person on an average diet about 30 gms. may be expected; for an invalid, about 20 gms.

In general the amount of urea is parallel to that of the nitrogen.

The urea may be diminished because the nitrogen is diminished, or is excreted in other forms, particularly as ammonia; for by far the most of urea passes through the ammonia stage, and one of the most important functions of the liver is that of changing the ammonia to urea; hence in certain cases of liver disease the output of urea diminishes and that of ammonia increases, constituting even 50 to 60 per cent. of the total nitrogen (see page 115). It is true, however, that in other cases with marked gross lesion of the liver the percentage is about normal. Again, the nitrogen may be eliminated as ammonia because of acids ingested or formed within the body and neutralized by it to protect the mineral alkali; hence it is withdrawn from urea formation. Such is true in diabetes and in cachexia, in which there is a disturbance of the carbohydrate absorption or use, thus forcing the body to use only the pure proteid of food or tissues,—that is, an acid producing diet.

A few words may at this point be said concerning the origin of urea. Its sources are: the direct hydrolysis of albumin, in which case among the precursors of urea have been found creatin, lysatin, and arginin, Drechsel claiming that 10 per cent. of the urea may have this source; it may be formed from amido-acids, leucine, glycocoll, asparaginic acid, as proved by the isolated liver, but how much this occurs in the body cannot be said. The great mass of urea is certainly formed from ammonia salts changed by the liver to urea. Amido acids, carbamic acids, and ammonium carbonate are probably the early stages in this process. Carbamic acid has been proved in the urine of man after the ingestion of large amounts of milk of lime (Abel and Muirhead),[23] and is probably a constituent of normal urine. These ammonia compounds, when not excreted, probably produce toxic symptoms, since their direct injection or their increase in the blood in a dog with an Eck fistula is followed by such symptoms. And, lastly, the possibility of an oxidation synthesis has been proved by Hofmeister. The liver is the only organ which has been proved to have this so-called function of neutralizing ammonia; that is, of changing a toxic end-product into a harmless one. In men with liver cirrhosis, acute yellow atrophy, and in phosphorus poisoning, from 50 to 60 per cent. of the nitrogen has been found as ammonia. In other cases, however, there is a normal percentage. It is true that in all such cases the possibility of the increase or formation of an acid may be sufficient to explain this output of ammonia.

That urea represents nitrogen needlessly consumed, hence that practically the whole animal kingdom overeats, we are not yet ready to believe.

Estimation of Urea.—Since urea is one of the bodies most influencing the specific gravity, an approximate estimate may be formed from this, but due allowance must be made for chlorides.

[23] Arch. f. Exp. Path. und Pharm., vol. xxxi.

Specific Gravity.	Per Cent. of Urea.
1014	1
1014 to 1020	1.5
1020 to 1024	2 to 2.5
1028	3

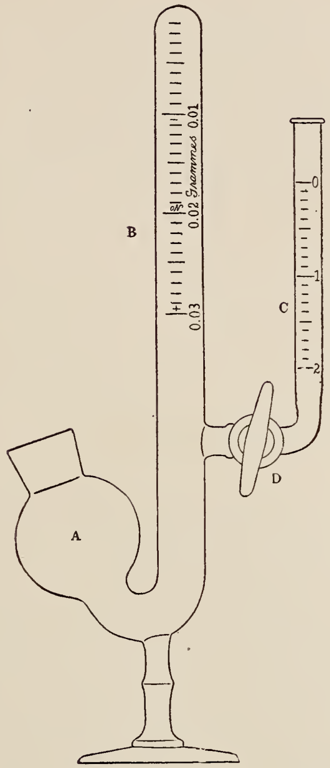


FIG. 20.—Heinz modification of Hüfner apparatus for urea determination. A, bulb; B, graduated tube to collect and measure the nitrogen; C, tube for urine; D, stopcock.

Hüfner's Method.—This method has been the most popular, and even now it can do very useful service; it depends on the decomposition of urea by sodium hypobromite, and the collection and determination of the volume of nitrogen thus liberated. The method is not accurate (see page 106). The simplest apparatus used is the Doremus tube shaped like the above figure, but without side tube and foot. The urine is measured in a 1 cc. pipette with rubber bulb and bent end. This is thrust, when filled exactly to the mark with urine, into the bend of the tube, mouth upward, and the urine slowly and steadily expressed into the urine. The tube is so tilted that all the gas will collect in the long tube. It must be remembered that an error of one drop in the amount of urine means an error of 6 per cent. in that of urea. The Hüfner apparatus allows of a very accurate estimation of the amount of urine used, a greater accuracy that the method justifies. Since the error inherent in

the method is so great, and the Hüfner apparatus large and expensive to use, we recommend the Heinz modification of the Doremus tube, which is simple, easy to use, and a sufficiently accurate application of this method. The tube (see Fig. 20, B) is filled to the prescribed mark with a solution of sodium hydroxide which contains 100 gms. per 400 cc. To this is added 1 cc. of bromine and the fluids mixed. It is then diluted with water until the vertical tube and the elbow are filled. This means a dilution of about one-half. The hypobromite solution may also be made as follows (Rice): Sol. a.—NaOH, 100 gms. dissolved in 250 cc. of water. Sol. b.—Bromine, 1 part; KBr, 1 part; water, 8 parts. Mix in equal parts at time of test. The small side tube C is then filled with urine. This is graduated, and one or more centimetres may be introduced into B. The large tube is graduated, and after all the gas has formed and risen and the foam subsided a direct reading of grammes of urea per cubic centimetre may be made. The changes in volume of gas due to temperature should be considered. This method is based on the formula $CO(NH_2)_2 + 3NaOBr = 3NaBr + N_2 + 2H_2O + CO_2$.

Notes.—The urine gives the best results when about 1 per cent. solution of urea is present. About fifteen minutes should elapse before the reading is made. The reaction takes place best at a temperature of 20° C. The carbon dioxide is dissolved by the excess of sodium hydroxide. About 8 per cent. of the nitrogen of urea is left in solution as NaOCN and $NaNO_3$, hence we do not read all, but this loss may be diminished by diluting the urea solution, concentrating the hypobromite solution, or especially by concentrating the sodium hydroxide, and also by using a temperature a little above 20° C. This loss is in normal urine almost exactly balanced by the nitrogen gained from other bodies. For instance, uric acid gives up about one-half of its nitrogen, also the xanthin bases; creatinin about one-third. Albumin must be removed, since this gives off some nitrogen and gives a precipitate which much hinders reading. Ammonia bodies are the great source of error, since ammonia gives off nitrogen as easily as urea. It is interesting that Eijkmann showed this point twenty years ago. Joslin[24] found in diabetes errors of almost 100 per cent.; *i.e.*, in one case 80 gms. of urea were found by this method when but 23.8 gms. of nitrogen were present. Dr. Slemons had similar experiences in this laboratory. Pflüger and Bohland advise to precipitate the urine first with phosphotungstic acid and hydrochloric acid to remove the extractives. The urine is then filtered and the filtrate used. In so doing, however, it should be remembered that those bodies which make up for the loss of the nitrogen of the urea have been removed, and hence the result is always somewhat (perhaps 8 per cent.) too low.

The Mörner-Sjoqvist.—This method was thought reliable for the determination of urea alone. Albumin must first be removed by heat and acetic acid, and then the original volume of urine restored. Five cc. of urine are placed in a flask with 5 cc. of a barium mixture (saturated barium chloride solution to which barium hydroxide is dissolved till 5 per cent.); 100 cc. of a mixture of 97 per cent. alcohol, 2 parts, and ether, 1 part, are then added. This is allowed to stand until the next day in a closed vessel. It is then filtered on a suction filter, preferably through a small filter paper about 10 cm. in diameter and into a Kjeldahl flask. The precipitate is well washed with alcohol and ether. It is sufficient to obtain about 50 cc. of filtrate. Urea will be practically the only nitrogenous body left

[24] Boston Med. and Surg. Jour., 1902, vol. cxlvii. p. 700.

in solution. The alcohol and ether are then evaporated off at a temperature not above 60° C., or distilled off at reduced pressure if a good suction pump is at hand. When about 25 cc. are left there is added a little water and burnt magnesium oxide and the evaporation or distillation continued until the distillate is no longer alkaline, which is true, as a rule, when only from 10 to 15 cc. are left; otherwise ammonia will be left in the filtrate. The fluid is then poured—and the flask washed—into a flask. A few drops of concentrated sulphuric acid should then be added, and it is then evaporated on a water-bath to a very small volume, since we wish to get rid of all the alcohol which will blacken the solution and foam badly. Twenty cc. of pure sulphuric acid are then added and one continues as in the Kjeldahl nitrogen determination.

The amount of nitrogen multiplied by 2.143 will give the weight of the urea. Hoppe-Seyler advises that when the fluid is evaporated to 10 to 15 cc. about 10 gms. of crystalline phosphoric acid be added, and one proceed as in the Schöndorff method.

A still better method is that of *Schöndorf*,[25] which gives results from 0.08 to 0.19 per cent. lower than by the Mörner. This method is based on experiments that phosphotungstic acid will precipitate all of the nitrogenous bodies excepting urea; it would precipitate this in a solution of over 3 per cent. urea.

Fifty cubic centimetres of urine (if the specific gravity be over 1017, it should be diluted) are measured in a closed graduated cylinder of 200 cc. capacity, and the acid mixture (phosphotungstic acid 10 per cent. 9 parts, HCl specific gravity 1.124, 1 part) added until full precipitation is obtained. The amount necessary for this should be determined by the following preliminary test. To 10 cc. of urine is added the phosphotungstic acid mixture from a pipette, stirring by shaking, until 1 cc. of the clear filtrate (filter repeatedly until clear) does not cloud when a few more drops are added. It is sufficient to get within 1 cc. of the necessary amount.

The cylinder is then filled up to the 150 or 200 cc. point with HCl (of sp. gr. 1.124, diluted ten times), well shaken, and allowed to stand for twenty-four hours. It is then filtered through a double filter until clear. The clear filtrate is rubbed up with $Ca(OH)_2$ until alkaline, and after the blue color disappears is filtered. Fifteen or twenty cubic centimetres (that is, the amount which corresponds to 5 cc. of urine) are then measured into an Erlenmeyer flask in which are put 10 gms. of crystalline phosphoric acid. This is heated in a dry chamber (a sand-bath) for four and a half hours, the time reckoned from the point when all the water is evaporated off, and at a temperature of 150° C. After cooling, the syrupy mass is dissolved in warm water, put into a distillation flask, and the further steps are the same as those of the Kjeldahl nitrogen determination. The amount of nitrogen multiplied by 2.143 will equal the weight of urea in 5 cc. of the urine.

Pflüger and Bleibtren advise that 5 cc. of the filtrate, after the addition of $Ca(OH)_2$, be used to make an ammonia determination, the nitrogen of which is

[25] Pflüger's Arch., Bd. 62.

subtracted from the result; but if pure phosphotungstic acid be used this is unnecessary since the ammonia will all be in the precipitate (Gullich).

In all methods it is to be supposed other bodies nearly related to urea are determined as well. It has been repeatedly shown[26] that if there be even 0.1 per cent. sugar in the urine there is a considerable loss of urea nitrogen, hence all must be removed by fermentation.

Folin Method.—To 5 cc. of urine in a 200 cc. Erlenmeyer flask are added 5 cc. concentrated HCl, 20 gms. $MgCl_2$, a piece of paraffin the size of a small hazel-nut, then 2 to 3 drops of 1 per cent. aqueous alizarin red. This flask, protected by a safety-tube, is boiled till each drop of reflow makes a very perceptible thump; the heat is then reduced and continued one-half hour. The contents of the flask must remain alkaline, hence when seen to turn red a little acid is allowed to flow back from the safety-tube. At the end of an hour the contents of the flask are transferred to a 1 litre flask with about 700 cc. water, then about 20 cc. of 10 per cent. NaOH, and the ammonia distilled till almost all over and the distillate not alkaline. The distillate is then boiled to drive off the CO_2, cooled, and titrated. The ammonia of the urine and of the $MgCl_2$ must be subtracted.

Using the Schlösing method, v. Jaksch[27] has shown that among patients in general, 83.93 to 91.07 per cent. of the total nitrogen is urea, and this constitutes from 95.85 to 98.36 per cent. of the nitrogen not precipitated by phosphotungstic acid; from 1.52 to 3.61 per cent. of the total nitrogen is in amido-acids, and from 5.16 to 8.51 per cent. of the nitrogen precipitated by phosphotungstic acid is in amido-acids and ammonia bodies. To double the amount of total nitrogen is a sufficiently correct way of clinically estimating urea; that is, he doubts any considerable disturbance of the distribution of nitrogen.

The amido-acids are increased in liver disease, in typhoid fever (the output may be even 0.5 gm. per day), in diabetes mellitus (even to 0.64 gm. per day), and in some cases of Graves's disease.

At this point we may suggest that simply because a person has a certain disease does not mean that that case will necessarily show the changes in nitrogen distribution usually ascribed to that disease; for organs in general are functionally very sufficient despite disease until the disease reaches a point rendering them functionally insufficient, then the characteristic changes may suddenly develop.

Halpern,[28] using similar methods, found in nephritis, carcinoma, and inanition a relative decrease of urea; yet this was not constant, in some cases finding normal figures, in others an increase of extractives and ammonia but not of amido-acids; in liver disease no relation between urea and the amido-acids, although the former fell; in blood diseases, leukæmia, severe pernicious anæmia, in tuberculosis, the distribution of nitrogen was normal. From work like that above mentioned we see that in the usual run of cases the various diseases studied do not disturb the nitrogen distribution in any char-

[26] Landau, Maly, Jahresb., vol. xxxiii.
[27] Zeitschr. f. klin. Med., 1903, vol. l. p. 167.
[28] Ibid., p. 355.

acteristic way. We must wait for observations on a series of more severe cases of these same diseases.

We give herewith a few of the *properties of and tests for* urea, since this is a most important body. Urea when pure occurs in crystals which are needles or prisms belonging to the tetragonal system; colorless, striated, pale, four-sided columns with ends of one or two oblique planes, and sometimes hollow. They contain no water of crystallization. It is not hydroscopic, and does not change in the air; it is decomposed by heat, the decomposition and the evolution of ammonia beginning at 100° C., but chiefly at 130° to 132° C.

The *furfurol* test is one of the most important. According to Schiff, one crystal, the size of the head of a pin, is brought in contact in a porcelain dish with one drop of concentrated aqueous solution of furfurol. At once is added one drop of hydrochloric acid (specific gravity 1.100) and one sees a rapid change of colors from yellow, to green, to blue, to violet, and in a few minutes a fine purple-violet color. Alantoin gives the same test, but less intense and slower. An old furfurol solution will also give the test without urea. Huppert advises the following method: 2 cc. of concentrated furfurol solution plus 4 to 6 drops of concentrated hydrochloric acid are mixed. The mixture must not stain red. To this is added one crystal of urea. In a few minutes is seen a deep violet color, which gradually becomes black, and then appears a black precipitate.

The *biuret* test is one of the best-known urea tests. Urea, if fused, gives off biuret and cyanuric acid. This occurs at a temperature of 100° C. To test this a few crystals are put in a dry test-tube and heated gently until fluid. This is then cooled, dissolved in water, made strongly alkaline with NaOH, and then 2 per cent. $CuSO_4$ solution added drop by drop. A beautiful violet color will result.

When only a crystal or so is at one's disposal, as, for instance, in the case of frost upon the skin, the best urea test is the *nitric acid* or *oxalic acid* test. Urea in the presence of concentrated nitric acid forms a compound, $CO(NH_2)_2$-HNO_3, in crystals, thin rhombs or hexagonal plates, which often overlap like shingles. They are colorless, and have acute angles. If they form slowly, large, thick, rhombic prisms are produced. These crystals heated volatilized without residue, an essential point in the test to exclude similar crystals of the heavy metals. No nitrous acid should be present in the nitric acid, since this in the cold will break up the urea, forming carbon dioxide, nitrogen, and water. To perform the test, one crystal or one drop of the concentrated solution is allowed to come in contact under the cover-glass with pure nitric acid. At the line of contact is seen the rapid formation of the above-described crystals. The urea must be in the concentration of at least 10 per cent.

Urea oxalate, $2CO(NH_2)_2H_2C_2O_4$, is less soluble in water than the nitrate, and hence this test is preferred by many. It is performed in the same way as the nitric acid test. The crystals are rhombs, hexagons, or plates. It is well to dissolve the urea in the least amount of absolute alcohol, and to use a concentrated ether solution of oxalic acid, or, better still, an amyl alcohol solution of both.

To *isolate urea* from any solution the albumin is first removed. The urine, *e.g.*, faintly acid is concentrated at a low temperature to a very small volume; nitric acid is then added in excess, the mixture being kept cool. The precipitate is filtered and pressed between filter paper. It is then dissolved in water and decomposed with barium carbonate, dried upon a water-bath, and the residue extracted with strong alcohol. The extract is decolorized if necessary with animal charcoal. Urea recrystallizes on cooling from the warm alcoholic solution. To determine it the Schöndorff method is applicable to albumin-free fluids.

Uric acid is a substance which has attracted an absurd amount of attention, and been the object of a great amount of careful work. The present status of opinion is that it is a specific oxidization product of the nuclein basis, and is increased only by an increase of these bodies

in the food, or an increased metabolism of tissue nuclei. Horbaczewski considered that this body is derived especially from the nuclei of leucocytes. Although this may in some degree be true, yet it probably explains but a small part. It is an interesting fact that in birds and certain reptiles the uric acid is the chief nitrogen compound of the excrementa; that in some carnivora (dogs and cats) it sometimes fails. In the herbivora it is always present, but only in traces, and in man it is present in a larger but still very varying amount. It has been shown also that the body has the ability to synthesize uric acid. If hypoxanthin be fed a patient, 50 per cent. will appear as uric acid. In the case of birds it is probable that just as in mammals the chief end product of nitrogen is urea, but this is synthesized to uric acid, while in mammals it is excreted unchanged; and that, lastly, if uric acid be fed to the body it will oxidize some of it; hence one is very wary in arguing from the amount in the urine to that found in the body. Recent work tends to show it an even more specific product of the nuclein bases than was supposed, and its output quantitatively related to these, although it is often delayed.

Uric acid, when pure, is a white powder of very small prisms or plates. It is difficultly soluble in boiling water and very little in cold. It is more soluble if not pure. Urea is its best solvent, and this in the urine can hold all the uric acid there in solution. It is insoluble in alcohol and ether; somewhat in hydrochloric acid and alkaline carbonates. The cold solution does not redden litmus. It reduces Fehling's solution when heated, but not bismuth solution. It is broken up by NaOBr, about 47.8 per cent. of its nitrogen being given off. The output may be said to vary normally from 0.2 to 1.25 gms., an average of 0.7 gm. in twenty-four hours, which represents from 1 to 2 per cent. of the total nitrogen. It is increased physiologically by an increase in the nucleins of the diet, sweetbreads being a favorite food to show this, since they increase it from 0.5 to 2 gms. in twenty-four hours. The maximum output occurs from three to five hours after a meal (that of the nitrogen in nine hours). There is a relatively large output in the newborn. In the adult the nitrogen of the uric acid is to nitrogen of the urea as 1 : 50 to 70, but in the case of the newborn as 1 : 13 to 14.

The amount varies considerably, particularly in different individuals. Burian and Schur have simplified the question greatly by showing that the uric acid output may be divided into two fractions,—the exogenous and the endogenous. By exogenous is meant the uric acid which is formed from the food directly; the endogenous, that part arising from the tissue proteid. This endogenous fraction is therefore the interesting fraction to consider, and in metabolism work involving it the patient should be on a diet—*e.g.*, of eggs and milk—which

covers his nitrogen and heat needs, but which does not contain nucleins.

Concerning the pathological variations there is the widest divergence of opinion, and hardly one claim is unchallenged. This is chiefly due to the fact that the difference between the endogenous and exogenous was not recognized.

The uric acid is pathologically increased when there is an increased proteid catabolism. Such is true of fever, in which case the increase is parallel to that of urea.

There is an absolute increase in leukæmia, the record being that of Magnus-Levy's case, with an output of 8 gms. in twenty-four hours. As a rule, it is about 2 gms., and the nitrogen of the acid is to the nitrogen of the urea as 1 : 9.

The relation in gout is still uncertain. One thing is quite certain, that during the quiescent interval between attacks the acid is below normal, rising to normal with the acute symptoms, then to sink again. This is of diagnostic importance in a suspicious case of arthritis. Whether it is retention of the acid or diminished formation followed by an increase is still to be settled, but the large accumulations of the acid in the tophi and around the joints is good evidence of an increased production; these patients do not respond as normally to an increased nuclein-rich diet by an increased uric acid output.[29] In rheumatism the question is still unsettled. In diabetes mellitus the increase is not marked, 2 to 3 gms., and is due to diet; in pernicious anæmia an increase is claimed. In pneumonia during resolution the output is increased, probably from the breaking down of the nuclei in a large exudate. In cirrhosis of the liver it is said to be very much increased, Chabrié even stating that in certain cases the maximum, even 8 gms., is excreted. This is rather interesting, since the liver is certainly an organ which can synthesize uric acid. The uric acid diathesis so emphasized by Haig is still in dispute. V. Jaksch thinks it exists, symptoms of hypochondriasis and increased uric acid output being the two features.

It is said to be diminished by a poor diet, in nephritis, during the acute attack of gout, in certain chronic diseases, and after large doses of quinine.

One point of interest is that when the alloxuric bases are increased the uric acid decreases in the same proportion.

Urates.—The possible urates are:

(1) Neutral, $M\overline{U}$, which do not occur in nature.

(2) The monoacid or biurates, $MH\overline{U}$, which are gelatinous or crystalline bodies, and the best illustration of which are the needles found in tophi in gout.

[29] Reach, Münch. med. Wochenschr., No. 29, 1902.

(3) Quadriurates, $MH\bar{U}\bar{U}$, which are easily split to $MH\bar{U}$ and $\bar{U}$ by water, heat, or acid. They are less soluble than the biurates. The urate sediment is supposed to consist of this. Many observers think Roberts's quadriurates are merely mixtures of sodium biurate and uric acid.

Tests.—The murexid test is the one commonly used. The uric acid is dissolved in two drops of nitric acid. This is evaporated carefully to dryness, the residue being a beautiful red. Ammonia is then added and the color changes to a purple red. Had NaOH or KOH been used in place of the NH_4OH, the color would be more of a blue or bluish-violet. The color disappears rapidly on warming, an important point to differentiate certain other bodies. The test is more beautiful if evaporation is done over a water-bath, and if the ammonia be not directly added but placed in a small glass under a bell-jar near the residue; also if but little uric acid be used. If the residue be not red but only yellow, too little nitric acid has been added. More should be added and the evaporation repeated.

Guanin, xanthin, epiguanin, will also give this test, but these are excluded if the substance used was insoluble in an excess of HCl. In confirmation the color should be bleached by further heating, and the Fehling's reduction test tried with the body.

Quantitative Determination.—Only approximately this may be done by the Heller's albumin test, underlaying the urine with two-fifths volume of nitric acid. A cloudy ring appears above the line of separation. If the ring comes before five minutes, uric acid is increased; if after five minutes, it is decreased. The urine must be albumin-free.

The most correct method is the *Ludwig-Salkowsky*. This method, as slightly modified by Schmoll, is as follows: 240 cc. of urine are precipitated by 60 cc. of magnesium mixture (100 gms. of $MgCl_2$ dissolved in water, plus NH_4OH, till it smells strongly of ammonia. To this is added NH_4Cl until the precipitate is just dissolved, and the whole made up to 1 litre in volume). The mixture is then filtered, 250 cc. of filtrate (equalling 200 cc. of urine) are used, and precipitated with from 10 to 15 cc. of silver nitrate solution (1 litre containing 26 gms. of $AgNO_3$ and enough NH_4OH to dissolve the precipitate. The volume is then made up to 1 litre. This should be kept tightly corked in a dark bottle). The urine is then filtered, the precipitate washed with distilled water, and then brought into suspension in a litre of water made just acid with HCl. Three to 4 cc. of $CuSO_4$ (10 per cent.) are then added and the mixture boiled. The silver salt is then decomposed by H_2S while hot and just acid. After entire decomposition it is boiled and filtered and the filtrate evaporated to 15 cc. Ten to 15 drops of HCl are then added, and it is allowed to stand from one to two hours or more. The crystals of uric acid are then filtered out on a very small filter, washed with water slightly acidified with HCl, not too long, so that at the end the total volume of wash water is not over 40 cc. and the nitrogen of the precipitate on the filter paper then determined by the Kjeldahl method. The result multiplied by 3 will be the weight of uric acid. Or, the determination may be made gravimetric. The crystallizing solution is allowed to stand over night, and then is collected in a Ludwig glass wool or asbestos filter with a ground glass stopper, which has already been

dried at 110° C. and weighed. The precipitate is washed upon this, washing at first with the filtrate and then with the smallest amount of water, then with alcohol, then with CS_2 and ether, dried and weighed.

This method, although the most accurate and one which with the small modifications can be easily finished at the end of half a day, is still too difficult and demands too elaborate an apparatus to justify its use in general clinical chemistry. In a case of gout all that is necessary is to know whether the uric acid be much diminished or not, and a slightly less accurate method will suffice. Folin's modification of Hopkins's method is recommended.

Folin's Method.—To 300 cc. of urine are added 75 cc. of a uranium acetate reagent (consisting of 500 gms. of ammonium sulphate and 5 gms. uranium acetate dissolved in 650 cc. of water; 60 cc. of 10 per cent. acetic acid are then added and the whole made up to 1 litre). This solution is to remove the phosphates and certain bodies not well understood whose presence would in certain pathological cases disturb the accuracy of the method. The urine thus treated is well stirred and allowed to stand five minutes, and then filtered through a double folded filter. From the filtrate 125 cc. are measured into two beakers, each volume representing 100 cc. of urine. Five cubic centimetres of concentrated ammonia are added to each and the solution set aside until the next day. The clear fluid is then decanted through a filter, the precipitated ammonium urate is collected on the paper and washed with a 10 per cent. solution of ammonium sulphate. It should be washed until the filtrate is almost chlorine-free. In testing the filtrate of the washing for Cl with $AgNO_3$, a little HNO_3 should be added. The filter paper is then pierced and the ammonium urate washed into a beaker, using about 100 cc. of water. Fifteen cc. of concentrated sulphuric acid are then added, and the solution titrated while still hot with a twentieth-normal $KMnO_4$, until the first blush of red is seen through the whole volume of fluid. This color need last but a few seconds. Each cubic centimetre of the reagent indicates 3.75 mg. of uric acid. A correction of 3 mg. per 100 cc. of urine it is necessary to add.

By a twentieth-normal $KMnO_4$ is meant one of such concentration that 1 litre would contain 0.05 gm. of available oxygen to oxidize the uric acid. Hence 1.576 gms. of recrystallized $KMnO_4$ are weighed into 1 litre of water. Since weighing is not sufficiently accurate, it is best to make a slightly more concentrated solution. This is boiled, which renders the solution more permanent. It is then titrated against a tenth-normal solution of oxalic acid (6.3 gms. per litre) or potassium tetraoxalate (8.41 gms. per litre). Ten cc. of the oxalic acid solution are diluted to 100 cc. with distilled water, and 15 cc. of concentrated sulphuric acid added to produce a temperature of about 60° C. The potassium permanganate is then added drop by drop until a uniform red color appears which lasts about thirty seconds. The permanganate solution is then diluted until 10 cc. of the oxalic acid require 20 cc. of the $KMnO_4$ solution for the end reaction.

It is interesting that at the beginning of the titration the red remains longer than later. This is due to the fact that the combustion of the uric acid is much promoted by the increased percentage of the sulphate of manganese. The color

is not permanent, owing to other reducing bodies, and the student, to use the solution satisfactorily, should have standardized it himself, that he may know what to consider an end reaction.

To get an oxalic acid sufficiently pure it is necessary to recrystallize two to three times a cold saturated solution; or, better, to recrystallize first from hot dilute HCl (10 to 15 per cent.), then from hot alcohol, then from water. The aqueous solution must be heated till the odor of ethyl oxalate passes off. Oxalic acid cannot be dried in a desiccator or hot-air bath.

In the above methods great care must be used to avoid error from the uric acid or urates which may have precipitated, and which must be redissolved by warming the urine, or by the addition of a little saturated lithium carbonate solution.

Rudisch and Kleeberg[30] have recently reported a method for determining uric acid and the purin bases which they think even superior to the Ludwig-Salkowski method in accuracy, and so quick a method that it can be used clinically. These bodies are precipitated by an excess of fiftieth-normal $AgNO_3$, and the excess of silver determined volumetrically by fiftieth-normal KI, the end reaction being recognized by testing, after the addition of the successive portions of KI, the mixture in test-tubes with nitrous-sulphuric acid (25 cc. H_2SO_4 to 75 cc. H_2O, then 1 cc. of fuming HNO_3) and starch solution, until the blue of starch iodine compound appears. The separation of uric acid and the other purin bodies depends on the solubility of the silver compounds of the latter in strong ammonia solutions. This method is still recent, and for the details the reader is referred to the original article.

The Purin Bases.—The purin, alloxuric, xanthin, or nuclein bodies, all shown to contain the purin ring, occur in the urine in very small amount. Their formation from the nucleins is represented by the following diagram:

Nuclein

Simple proteid — Nucleic acid

Nucleic acid: Thymic acid — Purin bodies

Thymic acid: Thymin, Uracil, Cytosin — Metaphosphoric acid

They are xanthin, guanin, hypoxanthin, adenin, heteroxanthin, paraxanthin, episarkin, epiguanin, methylxanthin, carnin. Of these ten bodies, the three which form the chief amount in the urine are heteroxanthin, paraxanthin, and methylxanthin, and these are derived wholly from the caffeine, theobromine, and theophylin of the food. Guanin and carnin are still unproved. The total amount occurring in the urine is from 15.6 to 45.7 mg. in twenty-four hours. Others consider 87 mg. an average output for a mixed diet (Camerer), 44 mg. for a meat and 111 mg. for a vegetable diet.

Xanthin occurs normally in the urine only in minute traces. More rarely it is the chief constituent of urinary sediments and calculi, several of which have

[30] Amer. Jour. Med. Sci., 1904, vol. cxxviii. p. 899.

been described. It is increased in leukæmia, nephritis of children, in which case there may be 28.5 mg. per 100 cc. instead of, as normally, 3.8 mg. From 10,000 litres of urine 16 gms. have been isolated.

The principal test of xanthin is *Weidel's.* The body in question is boiled in a test-tube with hydrochloric acid and a little $KClO_3$. It is carefully evaporated to dryness, and the residue moistened with ammonia. A red or a purple-violet color results. Another test is to evaporate to dryness in a porcelain dish with nitric acid, producing a yellow residue which, on the addition of NaOH and warming, becomes a purple red.

Guanin is claimed to occur in the urine, especially in leukæmia. It gives the same nitric acid test as xanthin, excepting that the alkali gives a more blue-violet color. It does not give the Weidel reaction.

Hypoxanthin is present in the urine and in considerable amounts in leukæmia. It gives neither the nitric acid nor the Weidel tests.

Adenin occurs in urine, especially in leukemia. The characteristic reaction is that if the crystals be warmed slowly in an amount of water insufficient to dissolve them, at 50° C. there appears a sudden cloud. It does not give the nitric acid nor the Weidel test. Its other reactions are the same as hypoxanthin.

The QUANTITATIVE DETERMINATION used for these bodies is usually that of Salkowski. From 400 to 600 cc. of urine (albumin removed) are precipitated with a magnesium mixture and filtered. The filtrate is then precipitated with a 3 per cent. ammoniacal silver solution (6 cc. per 100 cc. of urine). The silver precipitate is washed thoroughly. It is then brought into about 600 to 800 cc. of water, slightly acidified with hydrochloric acid and decomposed with H_2S. The fluid is then heated to boiling and filtered hot. The filtrate is evaporated on a bath to dryness and the residue extracted with 3 per cent. hot sulphuric acid, from 25 to 30 cc. being used. This extract is allowed to stand for twenty-four hours. The uric acid is then filtered out and washed, the filtrate made alkaline and again precipitated with $AgNO_3$. It is then collected on a small chlorine free filter, washed, dried, carefully ashed, the ash dissolved in nitric acid and titrated for chlorine by the ordinary Volhardt method. One part of silver equals 0.277 parts of the xanthin base nitrogen, or 0.7381 parts of the xanthin bases. The uric acid can be determined in the same portion.

The enormous literature on the xanthin bases has lost its value since the methods formerly used have been found incorrect, hence at present nothing can be said of the clinical value of these bodies. It is interesting, however, that in leukæmia these bodies have been found to be increased, also in tuberculosis; and that there seems to be an antagonism between them and the uric acid, so that while the sum of both remains constant, when one increases the other decreases, and *vice versa.*

Ammonia.—There is always in normal urine a small amount of ammonia, varying from 0.3 to 1.2 gms. (average 0.7 gm.) in twenty-four hours on a mixed diet. This represents from 3.5 to 5 per cent. of the total nitrogen. It reaches its maximum percentage during sleep—that is, when digestion is at rest. The presence of this ammonia in normal urine should not be forgotten. It could be ammonia withheld from urea formation to balance acid ions, but this may not explain all, since there is still ammonia present after a long continued alkaline medication.

Ammonia is one of the most important products of proteid metabolism. In the arterial blood there is 0.4 mg., and in the portal blood 1.85 mg. in 100 cc. (Hordynski). It is found in all the tissues, especially the stomach wall which contains 36.4 mg., and in the in-

testinal wall 32.4 mg., per 100 gms. of the organ, being especially abundant at the height of digestion. In the other organs there is a more constant amount. It is rapidly changed to urea, especially by the liver, and hence in certain cases of disease,—*e.g.*, cirrhosis and cancer,—with the total nitrogen unchanged, the percentage of urea will fall and that of ammonia rise. These ammonia bodies may be supposed to cause a certain toxæmia when increased, since injected ammonia compounds are toxic, and dogs with the Eck fistula manifest symptoms of toxæmia.

The relation of $N : NH_3$ is quite constant on a constant diet, and is not affected by the amount of proteid. Much fat, however, does increase the percentage of NH_3. During secretion of the HCl of the gastric juice the nitrogen per cent. rises.[31]

Ammonia is increased by the ingestion of inorganic acids and of organic acids which cannot be further oxidized, and by those which arise in the body, man and carnivora thus protecting their native alkalinity against depletion in acid intoxication. The herbivora cannot protect themselves as well, and hence suffer more quickly. Such acids may arise in considerable amount in the normal body if the diet be strictly proteid. It is increased in oxygen starvation; in fever, during the febrile stage and continuing into the convalescence (Rumpf); in diabetes, in which case oxybutyric and perhaps diacetic are the acids present; ammonia may be present in diabetes in from 8 to 12 gms. in twenty-four hours and represent from 25 to 40.4 per cent. of the total nitrogen. In a case of periodic insanity Edsall found a marked reduction just before the attack, and a rise as the attack came on. In certain cases of liver cirrhosis the ammonia is increased, since the liver fails to form urea.

Dr. Williams has put the determination of ammonia to very practical use in his obstetrical wards of this hospital. In cases of the pernicious vomiting of pregnancy the percentage of ammonia is much increased, even to 20 to 45 per cent., while in the cases of nervous vomiting, or reflex from the pelvis, and in eclampsia, it is not. With this very high ammonia percentage the urine need show no casts or albumin. Definite hepatic lesions are found. If this high ammonia percentage is found, the uterus is emptied, and the ammonia drops at once. In a normal pregnancy the ammonia percentage is somewhat increased, reaching a maximum during labor.

Determination.—The Schlösing method is the one commonly used. (See Fig. 21.) This is simple, and yet is not perfectly satisfactory, since the results are somewhat too high. Twenty-five cc. of urine are mixed with 10 cc. of milk of lime. The broad vessel, B, in which this is placed, is at once covered over by a bell-jar, under

[31] See Schittenhelm, Deutsch. Arch. f. klin. Med., 1903, Bd. 77, p. 517.

which have been previously put 20 cc. of tenth-normal H_2SO_4. The bell-jar is then well greased, to render it air-tight, and allowed to stand for from three to four days, during which time the milk of lime will have set free all of the ammonia which the sulphuric acid then takes up. It is well that the sulphuric acid dish, C, rest upon the dish containing the urine. At the end of the three or four days the sulphuric acid is titrated against tenth-normal sodium hydroxide; the number of cubic centimetres multiplied by 1.7 mg. equals the weight of ammonia in 25 cc. of urine. If any moisture is present on the inside of the bell-jar the reaction of this should be tested, and if alkaline the entire interior of the bell-jar should be washed into the sulphuric acid before titration.

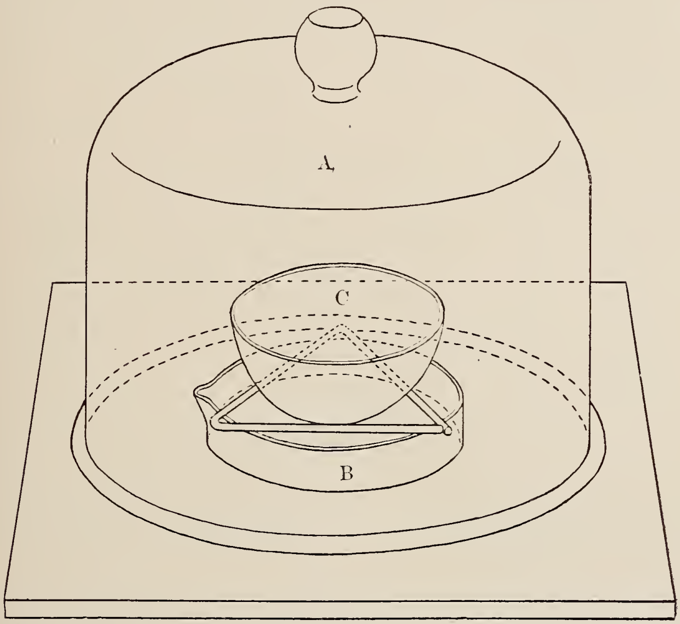


FIG. 21.—Ammonia determination, Schlösing method. A, bell-jar; B, dish containing urine; C, dish containing acid.

The modifications proposed by Schäffer, working under Folin's directions, are the following: To 25 cc. of filtered urine are added 0.5 gm. of sodium carbonate plus an excess of sodium chloride. The sodium carbonate will not split off ammonia from any of the other nitrogenous compounds, as for instance urea, and the sodium chloride will prevent decomposition. The urine should be placed on a dish from 15 to 17 cm. in diameter that the layer be not over 2 mm. deep; a wide crystallizing dish or a wide Petri's dish is the most satisfactory. The time may be reduced to forty-eight hours (not less) if the apparatus be kept at 38° C.

Many other methods have been proposed which have given a certain amount of satisfaction. One of the best known is the Nencki, which, as modified by Steyrer, is as follows (see Fig. 22):

Steyrer's Method.—From 20 to 30 cc. of urine according to its concentration are placed in a flask, A, through the cork of which extend three tubes. The

one of these, F, is connected with a sulphuric acid wash-bottle, D, and provided with a stopcock, E, that the in-flow of air may be regulated and that ammonia may be excluded from the apparatus. Through the second perforation is introduced a separating funnel, G, through which may be added the 50 cc. of milk of lime. The third tube, H, connects this flask with the interior of another, B, and is bent at its extremity, that it may reach to the bottom of this flask and therefore be well covered by the 50 cc. of quarter-normal sulphuric acid therein contained. The interior of this flask is connected with a strong water-pump, J. It is well that it be protected by a Wolff bottle, C, to prevent the loss of any sulphuric acid. After the urine has been introduced and all is ready, the suction is begun, and the 50 cc. of milk of lime are allowed to enter. The vacuum is controlled by the stopcock at the farther end of the apparatus and maintained at from 18 to 25 mm. of mercury. In one hour all the ammonia will have passed over. The flask of urine is immersed in a water-bath held at 36° C. Steyrer considers that urea is not decomposed by milk of lime at this temperature. In case the urine contains albumin, it is recommended that some alcohol be added to prevent the foaming. The use of milk of lime has been severely criticised, since a certain decomposition of urea is said to occur. Magnesium oxide has the disadvantage in that the formation of ammonium magnesium phosphate crystals withdraws a certain amount of ammonia from determination.

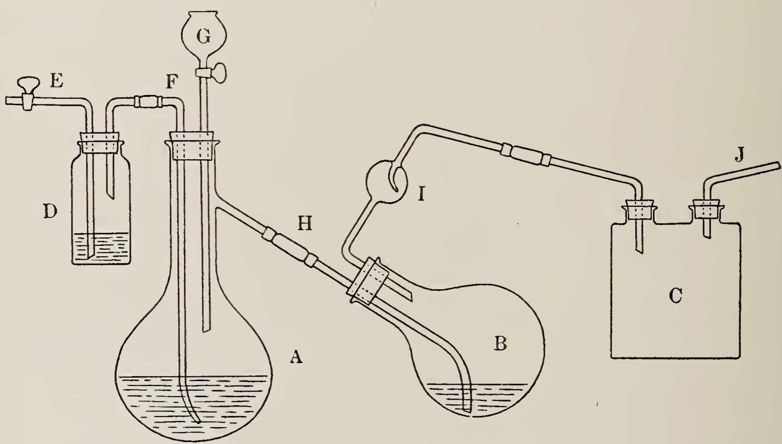


Fig. 22.—Ammonia determination, Steyrer's apparatus. A, flask for urine; B, flask for standard acid; C, Wolff bottle to catch acid which may be aspirated from B; D, sulphuric acid wash-bottle through which all air passes; E, stopcock to regulate air-inflow; F, tube connecting D and A; G, funnel for milk of lime; H, tube connecting A and B; I, safety-bulb; J, tube to air-pump.

Folin's Method (Fig. 23).—To 50 cc. of urine in flask, A, are added 15 to 20 gms. of sodium chloride and 50 cc. of methyl alcohol. In B (45 cm. high and 5 cm. wide) are placed 25 or 50 cc. of tenth-normal H_2SO_4, and in C some of the same acid diluted with a small amount of water. If too much water be added, there is danger of loss of acid by jumping during the violent commotion which is set up in the acid by the rapid passage of the vapor. Should such a loss occur, the acid can always be recovered by rinsing out the flask, D. When ready, 1 gm. of dry sodium carbonate is added to the urine in flask, A, the stopper quickly put in place, and suction started. With a good pump the pressure will be reduced to 10 mm. in two or three minutes. The liquid in the water-bath is maintained at 50° C. The boiling is allowed to continue for fifteen minutes. The acids are then titrated and the ammonia calculated. He recommends alazarin red 1 per cent. aqueous solution as indicator.

In none of the titrations should phenolphthalein be used as indicator, since this fails for ammonium salts. Among those which may be used are alazarin red, cochineal, and a dilute solution of hæmatoxylin which is used by Steyrer and seems very satisfactory.

Creatinin, the aldehyde of the creatin of muscle, occurs in the urine; creatin does not. In general its origin is the muscle of food and of the body. Its excretion is roughly parallel to that of urea; it is increased by a meat diet, and in hunger diminishes and even disappears. Sucklings have none in the urine until their diet is changed. It is probably increased by an increased metabolism of the body muscles. The relation between its output and muscular work has been much disputed, some claiming that it is increased only by excessive muscular work; others (Edsall) that it is increased by muscular exercise, and diminished in extensive muscular paralysis and in patho-

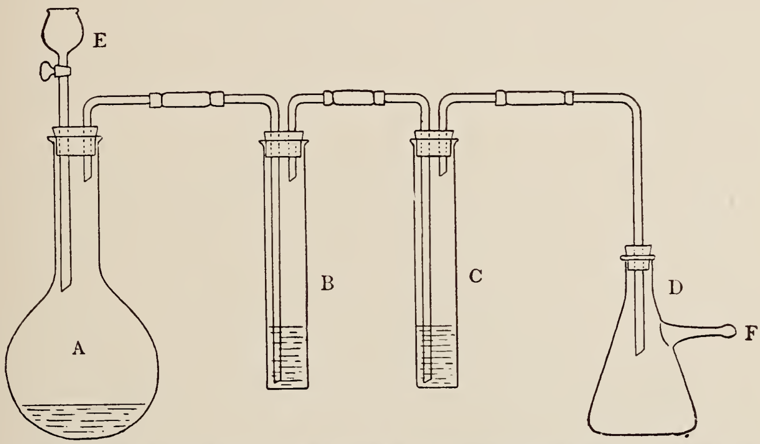


Fig. 23.—Ammonia determination, Folin's apparatus. A, flask for urine; B and C, bottles for acid; D, safety-bottle; E, funnel; F, outlet to pump.

logical conditions associated with a marked decrease in the function of the muscles; that while it is not a perfect index of the condition of the muscular metabolism, since it presents variations independent of the muscles, yet it is the index nearest the truth. In disease its relations are very little known. Normally the output is from 0.6 to 1.3 gm. per day, with an average of 1 gm. It has been found increased in acute fevers of all kinds, in diabetes due to the diet, in one case reaching 2 gms. per day; and in anæmias and cachexias. At present its determination is of no value, although good authorities now think that it contains most of the nitrogen which the body really used.

Jaffé's Test.—To the urine is added a little aqueous solution of picric acid and a few drops of dilute NaOH. An intense red color appears at once at room temperature and, increasing, remains for

hours. If acid be added it becomes yellow. Acetone should be removed by boiling, since it gives a more reddish-yellow color and is much fainter. Glucose gives on warming a red color. The test is positive in solution of 1 to 5000.

Weyl's Test.—To the urine are added a few drops of very weak sodium nitroprusside (sp. gr. 1003), then a few drops of weak NaOH. A ruby-red color appears which soon changes to yellow. Acetone will give a similar test and should be removed by heating, but the acetone urine would, if acetic acid be added, become of a cherry-red or purple-red, while in the case of creatinin the solution after adding acetic acid and heating becomes green and then a Berlin blue. The test is positive for 0.6 gm. per 1000.

The most important compound of creatinin is the zinc salt $(C_4H_7N_3O)_2ZnCl_2$. Creatinin is a reducing body, reducing Fehling's after long boiling to a colorless solution, and after still longer boiling, if an excess of copper be present, precipitates $Cu_2(OH)_2$. Creatinin, therefore, disturbs the copper sugar tests since it is a reducing agent, and, more important, it holds the $Cu_2(OH)_2$ in solution. Bismuth is not reduced.

Quantitative Determination.—Salkowski's modification of Neubauer's method. The urine must be albumin- and sugar-free. Two hundred and forty cc. of urine are measured in a graduated cylinder, made faintly alkaline with milk of lime, carefully precipitated with calcium chloride, and the whole then filled to 300 cc. Others recommend 480 cc. of urine, in which case all the succeeding figures must be doubled. This mixture is allowed to stand for fifteen minutes. It is then filtered through a dry filter until 250 cc. of filtrate (which equal 200 cc. of urine) are obtained. This is neutralized or made very faintly acid with hydrochloric or acetic acid, and then evaporated to about 20 cc., first over the free flame and then on a water-bath. The fluid is neutralized with soda, stirred up with an equal amount of absolute alcohol, and then washed with alcohol into a measuring flask holding 100 cc. and already containing some alcohol, the porcelain dish being washed well with alcohol and this added to the flask. The flask is shaken up well, perfectly cooled, shaking it a little from time to time in order to allow the escape of air, and then filled to the 100 cc. mark with absolute alcohol. It is allowed to stand for twenty-four hours, then filtered through a dry paper. Of the filtrate 80 cc. (equalling 100 cc. of urine) are mixed in a beaker with from 0.5 to 1 cc. of an alcoholic, absolutely acid-free $ZnCl_2$ solution, specific gravity 1.2. The beaker is then covered with a glass plate and allowed to stand from two to three days in a cool place. The precipitate is then collected on a small dry weighed paper, washed with the filtrate, then allowed to drip perfectly; the crystals are washed with as little alcohol as possible until no more chlorine reaction is obtained, and dried at 100° C., and then weighed. One hundred parts of the precipitate of the creatinin zinc chloride equal 62.44 parts of creatinin; instead of weighing, the creatinin may be determined volumetrically by determining its nitrogen.

Folin has published a colorimetric, quantitative method based on Jaffé's test.[32]

Oxyproteinic and Alloxyproteinic Acids.[33]—The first of these bodies was isolated by Gottlieb and Bondzyński, and the latter by Bondzyński and Panek. Although these bodies have not been sufficiently studied as yet, and already some have been unable to confirm this work, yet their presence in normal urine is claimed

[32] Am. Jour. of Insanity, 1905.

[33] Bondzyński and Panek, Ber. d. d. chem. Gesell., 1902, vol. xxxv. p. 2959.

to be sufficient in amount to explain all or quite all of the neutral sulphur, which renders them very interesting. These writers, however, also think the oxyproteinic acid explains Ehrlich's Diazo reaction (see page 151). Their sulphur content is about 6 per cent. They stand the nearest to proteid of all the products of proteid metabolism, and yet give none of the proteid reactions. In amount of the alloxyproteinic acid are excreted about 1.2 gms. per day, the oxyproteinic acid in about three times that amount.

THE INORGANIC ACIDS AND BASES

The Chlorides are one of the most important groups of solids in point of amount found in the urine. Measured as sodium chloride, there are excreted in twenty-four hours from 10 to 15 gms., seldom more. Chlorine is present in inorganic salts, the little claimed in organic compounds being much disputed, but with the weight of evidence against it.[34]

The source of the chlorides is the food. The amount excreted depends in the first place upon the amount ingested. Starvation will reduce them to a trace. More is excreted during the day than during the night. Chlorine is increased by increasing the water output and by active exercise. It is diminished from loss of fluid by diarrhœa or by vomiting; also by transudate and exudate formation, and increased as these fluids are reabsorbed, provided the absorption be rapid. In fevers it is diminished in a remarkable way, especially toward the crisis, following which, its increase, Sahli considers, is as important a sign of improvement as the lowering of the temperature; in pneumonia some think the rise may be the first sign of improvement; its entire absence a serious sign. A great diminution or absence of chlorides in the urine in a doubtful fever strongly suggests pneumonia. After the crisis the output soon returns to normal. The explanation is not clear. The drop is not due to the diet, since an increase in the amount of chlorine ingested is not followed by a corresponding rise, as normally. Among the reasons given are, that during fever the catabolism is of those proteids poor in chlorine; but it is found that chlorides per mouth or injected subcutaneously are also retained in the body; others say that they are retained in the exudates present, or again with retained water; but the retention of the water is itself a much disputed point. Sahli considers that all these factors are present. A great deal of experiment has recently been done. There certainly is a definite retention of chlorides, but the reason is not the lack of absorption nor the food. The chlorine is not increased in the blood, but is accumulated in the other fluids of the body and in the tissues, being increased in the tissues of some cases with marked renal insufficiency to even four times the normal (Achard and Laubry). Van der Bergh[35] explains it as an attempt of the blood to maintain its osmotic tension, there

[34] Ville and Moitessier, Compt.-rend. Soc. de Biol., liii. p. 673.
[35] M. J., vol. xxxi.

being an accumulation of the products of metabolism in the plasma due to a slight insufficiency of the kidney, which increases the osmotic tension of the blood, hence the chlorides do not enter the circulation, but remain fixed in the tissues. After convalescence has begun there is a sudden return to normal which Achard and Laubry name a "chlorine crisis." The sulphates and the phosphates do not return to normal at the same time. To these chlorine crises is attributed a prognostic value.

We have examined the records of thirty-four cases of pneumonia in this hospital. It is our routine in almost every case of pneumonia (all on a pure milk diet, 1500 cc. q. d.) to determine the total amount of the chlorides daily. Six of these cases were with crisis. In two the chlorides showed a drop toward the crisis. In one case the crisis was preceded by a rise. In the other cases the rise began with, or even four or five days later than, the fall in temperature. In these very few cases it will be seen that we obtained very little prognostic value from the determination of the chlorides. In no case were the chlorides entirely absent. The average on the day before the crisis was 1.3 gms., varying from 0.7 to 2.1 gms. The greatest rise began on the fifth day after the crisis, on which day it varied from 3.8 to 4.9 gms.

Of twenty-two cases of lysis, in seven-tenths of the cases there was a drop toward lysis. In two-tenths the chlorides began to rise one to two days before the temperature began to fall. On the first day of the lysis in ten cases there was above 1 gm., an average of 2.6 gms., and in one case 9 gms. In three cases they were absent before defervescence, and in two cases during the fall of temperature, hence in these cases entire absence was not a bad sign. They were lowest during the drop in one-third, and just before the temperature began to fall in two-thirds of the cases. They began to rise with the lysis in just one-half of the cases. The chief rise began after the temperature had reached normal. It was then rapid.

In five fatal cases the chlorides fell steadily until the end in three and rose in one. In one case death was preceded by six days of entire absence of chlorides.

In one case of delayed resolution the chlorides were interesting. Nineteen determinations were made during a period of twenty-two days. The lowest amount was 4.3 gms., and this occurred after the lysis. For the most part they varied from about 5 to 10 gms. per day, hence in this case there was comparatively little retention.

In those cases in which the fall in temperature is succeeded by several days of very slight fever the chlorides do not rise until the temperature is about normal. In cases with a normal temperature but with a continuous slight leucocytosis they did not rise until this had fallen below 10,000.

After chloroform inhalation the chlorides are increased. In diabetes insipidus there is a marked increase with the polyuria. In all chronic diseases there is a decrease which may be due to disturbed absorption, or to the diet, or to the condition of the kidneys.

In gastric disease the chlorine is diminished when there is considerable vomiting; when absorption is diminished, as in malignant pyloric stricture; and when lost by lavage or diarrhœa.

In chronic diseases, if the output becomes as low as 2 gms., and the diet cannot explain this drop, it is an ominous sign, and the cessation of chlorine one of oncoming death. It is said to aid in the differen-

tial diagnosis between meningitis, in which the output is very low, and typhoid, where it is only moderately low. There is a marked diminution in cholera, pyæmia, puerperal fever, and acute articular rheumatism. In cirrhosis of the liver it is said to be increased.

The retention in nephritis has attracted especial attention, particularly in view of the recent work of Widal and others concerning œdema. Their explanation is that, given a slight renal insufficiency, there may be a specific retention of chlorides, the output of other solids remaining normal. These chlorides are retained by the tissues and there retain water, thus leading to œdema. By "chloruræmia" is meant a partial renal insufficiency for chlorine elimination, with a rapidly developing general œdema, low Cl output, and increased albumin in the urine. It is rather hard, on this basis, to explain the absence of œdema after even a week of total suppression of the urine due, *e.g.*, to calculus, or in those cases in which at operation the only functioning kidney is removed. The injection of physiological salt solution does not seem to cause œdema (perhaps since so dilute), and seems even to improve the condition of the case (Ferrannini), but if increased albumin, slight hæmaturia, and sometimes uræmic convulsions follow the injection immediately, we cannot consider the injection harmless. We have repeated this work with varying success, but with none if the water intake be also controlled. This amount of salt makes the patient very thirsty and he consumes much more water. Achard and Loeper found that if 10 gms. of sodium chloride be given per mouth in acute nephritis, little or none of the ingested chlorine is excreted, the chlorides remaining low, from 1 to 2 gms. per day. In subacute nephritis with 4 to 10 gms. before the dose there is a slight increase, while in interstitial nephritis with 2.8 to 3.4 gms. output the most of that given is excreted. In uræmic conditions there may be little or none excreted.

Estimation.—A rough estimation of the amount of chlorides is made in the following way: To a test-tube of clear urine which contains no albumin 10 drops of pure nitric acid are added and then one drop of $AgNO_3$ (1 : 8). If the chlorides are normal or increased the precipitate is a compact ball which sinks to the bottom. If diminished, this ball is less compact; if much diminished, until only a cloud is produced without solid flakes. If the last be true, that is, a cloud merely, it means a chloride content of 0.1 per cent. or less.

Quantitative Determination.—The best method is Arnold's modification of Volhardt's method. With the chlorides are estimated also the minute trace of cyanides. The principle upon which the test rests is the precipitation of hydrochloric acid by silver nitrate in a solution made strongly acid by nitric acid. An excess of silver chloride is added, and after the precipitate is filtered out the excess of silver is determined by titration with ammonium sulphocyanate. The urine should contain no nitrites, and most observers add also, no albumin or albumose, since these are precipitated as silver albuminates. If albumin be present, it may be necessary to ash the urine (Neubauer's method). Hammarsten recommends that the albumin be removed by boiling with a trace of acetic acid. If this be done, however, the precipitate must be washed for some time in order that the abundant chlorides retained in the precipitate may be regained.

Solutions necessary:

(1) $AgNO_3$. 1 cc. equals 10 mg. of NaCl. The pure crystalline $AgNO_3$ is used, 1 litre to contain 29.075 gms. of the salt.

(2) Cold saturated solution of iron ammonium alum, or ferric sulphate, chlorine free (50 gms. of Fe_2O_6 per litre).

(3) HNO_3. Specific gravity 1.2, chlorine-free. If chlorine be present the acid should be distilled. The nitrous acid should be removed by urea.

(4) An ammonium sulphocyanate solution, 10 cc. of which will equal 10 cc. of the silver nitrate solution. To obtain this, 12.9 gms. of the NH_4SCN are weighed and dissolved in a little less than one litre of water, and well mixed. Twenty cc. of the silver nitrate solution, 5 cc. of the iron alum, and 4 cc. of nitric acid are mixed in a flask and then diluted to 100 cc. The ammonium sulphocyanate solution is then added from a burette. The first precipitate is brown, which at once gives place to a white precipitate of silver cyanate; the brown ferric cyanate remains only after the last particle of silver has been precipitated. The end reaction is very sharp. The solution should then be diluted the necessary amount and the fluid again tested to make sure that 10 cc. of the silver nitrate solution equals 10 cc. of the ammonium sulphocyanate. Others recommend (v. Jaksch) that this latter solution be so made up that 25 cc. will equal 10 cc. of the silver nitrate, while others that 20 cc. equal 10 of the silver nitrate.

In Arnold's method 10 cc. of urine are carefully measured with a pipette into a flask on the neck of which is a 100 cc. mark. Then are added 20 to 30 drops of nitric acid and 2 cc. of the iron alum solution. If necessary a few drops of 8 per cent. $KMnO_4$ are added until all red color disappears. The silver nitrate solution is then slowly run in, constantly shaking the flask until one is sure that all the chlorine has been precipitated and that there is an excess of silver. The flask is then allowed to stand for about ten minutes and then filled to the 100 cc. mark with water. This should then be mixed very thoroughly. There should be an excess of iron, otherwise the nitric acid can decolorize the ferric cyanate, but this excess of iron causes a brown rather than a red color in the end reaction. It is usually safe to add 20 cc. of the silver solution, while others recommend that 15 cc. be used. In general, a considerable excess gives the best results.

After the observer is sure that the contents of his 100 cc. flask is thoroughly mixed, it is then filtered through a dry filter until 50 cc. of clear filtrate are obtained. This is titrated with the ammonium sulphocyanate solution until the end reaction. The amount used indicates the excess of the silver solution in 50 cc. of filtrate. This amount multiplied by 2, since only one-half of the filtrate was used,

and subtracted from the number of cubic centimetres of silver nitrate originally added, will give the number of cubic centimetres of silver nitrate actually precipitated by the chlorides of the urine. This multiplied by 10 mg. will give the weight of the chlorine as sodium chloride in the amount of urine used.

Some add the iron alum solution to the 50 cc. of filtrate, not before. A much-jaundiced urine should be decolorized by adding a few drops of potassium permanganate and nitric acid. The urine is then warmed, allowed to stand for a few minutes, and filtered.

Lütke Method.—In this method a tenth-normal silver nitrate solution is obtained by dissolving 17.5 gms. of the silver nitrate in about 900 cc. of 25 per cent. nitric acid. To this is added 50 cc. of a 10 per cent. iron alum solution. The whole is then diluted to exactly 1 litre with water. A tenth-normal ammonium sulphocyanate solution is obtained by dissolving 7.6 gms. of the salt in a little less than a litre of water and then titrating this against the silver nitrate, thus determining its present strength, and then diluting, that 10 cc. of the one may equal 10 cc. of the other. Since the silver solution is weaker, at least 25 cc. is usually the amount used to give an excess. This method has the advantage of combining the three solutions in one. The mathematics involved is a disadvantage, since 1 cc. of the silver nitrate solution equals .00585 gms. of NaCl.

The *Purdy method* cannot compare for accuracy, and is almost as hard.

To 10 cc. of urine in a graduated 15 cc. centrifuge tube are added 15 drops of HNO_3, then $AgNO_3$ solution to the 15 mark. These are well mixed, then centrifugalized for three periods of five minutes each at 1500 revolutions a minute. The percentage of chlorides may then be read, using a table of values given in the last edition of his book. This volume of the precipitate is then by no means the smallest, as a little longer centrifugalization will show.

Phosphates.—Phosphoric acid occurs in the urine of man in considerable amount, and is often encountered as the precipitate in an alkaline urine, a constituent of some of the most common crystals, and the principal ingredient of some of the commonest stones. In addition to the mineral phosphate there is always a little phosphorus in organic combination.

The amount weighed as P_2O_5 in the urine of an adult is from 1 to 5 gms. in twenty-four hours, with an average of about 3.5 gms. The earthy phosphates are estimated as 1 to 1.5 gms., the alkaline from 2 to 4 gms. It varies chiefly with the food, especially with its content of calcium and magnesium, since these in the intestines form insoluble phosphates, which are little absorbed, hence the output may be less than one gramme. It is for this reason that in certain of the herbivora phosphoric acid is present only in a trace. It is important in metabolism experiments to control the diet carefully, that one may be sure

that an approximately constant amount will be absorbed. Ehrström considers, however, that the calcium of the food is not as important as early studies led one to believe, and thinks that acid calcium phosphate can be absorbed in considerable amounts. Nevertheless, from the stools he could recover from 12 to 50 per cent., an average of 30 per cent., of the total phosphorus ingested.

The phosphates are increased by an increased metabolism of the body tissues, and also by a nuclein-rich diet. The amount from this source, however, is small. They are increased by hard muscular work. In starvation the phosphorus falls a little, yet more is excreted relative to the nitrogen, and in this condition the relative value, that is P_2O_5 divided by N, equals 0.18. Ehrström found that the phosphorus was not excreted parallel to the nitrogen. In dogs on a pure meat diet the nitrogen is to the phosphoric acid as 8.1 : 1.

Clinically the phosphates have been the subject of much discussion. They are stated to be increased in extensive disease of bones, that is, rickets, osteomalacia, diffuse periostitis, and others; concerning each of these diseases, however, there is a great dispute: in destructive disease of the lungs, especially early tuberculosis; this group of cases also is open to considerable doubt, the coincidence of disease and increased phosphoric acid output being considered accidental. The same may be said of extensive disease of the nervous system. In mental disease Folin and Shäffer [36] found that during the periods of excitement the relative amount of phosphoric acid was diminished, but absolutely there was little change. They consider that the phosphorus metabolism of the brain is disturbed on the excited days, and that there is a compensatory increase on good days. It is also increased in meningitis, yellow atrophy of the liver, in diabetes mellitus and insipidus, after the use of chloral, KBr, and lastly in phosphorus poisoning.

They have been found diminished in acute diseases, for instance in pneumonia, during the height of the fever; this is true especially of the earthy phosphates, which point Gouraud considers may aid in the differential diagnosis between tuberculous processes, in which case the earthy phosphates are increased, and pneumonia. At the crisis comes a sharp rise, but one not always simultaneous with the rise in nitrogen and chlorine, while the ratio between the earthy and the total phosphates increases considerably. In one case of typhoid fever the total P_2O_5 rose after defervescence from 1.5 to 13 gms. The output of phosphoric acid in fevers is not at all parallel to that of chlorine, and there occur sudden large outputs which are independent of the diet (v. Jaksch has found, however, that in the acute lobar pneumonia of children there may be increased phosphoric acid).

[36] Amer. Jour. Phys., vol. vii. p. 135.

It is diminished in most chronic diseases; in all renal diseases, due it is supposed to the renal insufficiency, Purdy stating that the diminution in phosphates is almost as constant a feature as albuminuria; pregnancy, in which case it is attributed to the fetal bone formation; gout, in which disease the line of phosphoric acid runs quite parallel to that of uric acid. Certain cases have been reported in which, without any sugar output but with all the symptoms of diabetes mellitus, there is a phosphate excretion of even as high as 10 gms. in twenty-four hours. Such are cases of the so-called "phosphatic diabetes." To deserve this name the output should be at least 3.5 to 4 gms. per day. Others do not agree with Teissier that these cases resemble diabetes, but say neurasthenia. In some cases this is simply a temporary absence of the sugar which later appears, and as the phosphates fall.[37] A relative increase, formerly also passing under this name, in which P_2O_5 : N : : 17 to 20 : 100, occurs in malnutrition and starvation.

(For phosphaturia, see page 97.)

The organic phosphorus has been found by Mandel and Oertel not to be influenced by a phosphorus rich diet. They considered that its output is a good index of tissue catabolism.

There are four groups of phosphate salts,—the diacid, monacid, normal, and basic,—the salts varying in solubility in the order in which they are stated, the diacid being the most soluble. The monacid salts of calcium and magnesia are precipitated when the urine is made alkaline. On heating the urine, a flocculent precipitate of the normal salts is often seen (basic, v. Jaksch), which must not be confused with the albumin cloud. It has been found that this precipitate on heating is always the calcium phosphate with a trace of CaOx and $CaSO_4$, but never magnesium, since the calcium salts are more insoluble than the magnesium salts.

In leukæmia White and Hopkins[38] have found an absolute and a relatively (to nitrogen) diminished output, and they suggest a retention of the phosphorus in the blood to build new leucocytes. In the new-born the proportion between nitrogen and phosphoric acid is from 5 to 8 : 1.

Of the normal phosphates that of greatest interest is the $MgNH_4PO_46H_2O$ in the beautiful coffin-lid crystals of triple phosphate, which occur in all alkaline or amphoteric urines containing enough ammonia.

The acidity of the urine, although due to many acid components and to an unknown degree to each, is, however, chiefly due to the phosphates. Normally 60 per cent. of the phosphoric acid is present

[37] See Ralfe, Lancet, March 5, 1887.
[38] Journal of Physiology, vol. xxiv. p. 42.

as diacidphosphate, and 40 per cent. as the monacid salts, but the former varies from 34.9 to 74.2 per cent. In general it may be said that the urine is amphoteric if the diacid salts are from 30 to 50 per cent. and the monacid from 70 to 50 per cent. of the whole.

A common test, allowing an approximate determination of the phosphates, is made by filling a test-tube half full of filtered urine, adding ammonia, warming, and then allowing it to stand. If in from eighteen to twenty-four hours the deposit is from one-fourth to one-half inch deep the amount is normal, if less it is diminished. This is a precipitate of earthy phosphates. These are then filtered away, all of the filtrate put in the test-tube, and one finger's breadth of magnesium mixture added. The urine is then warmed and the precipitate of alkaline phosphates allowed to settle. If during the same length of time the sediment is from one-half to three-fourths inch deep the amount is normal.

The urine may be cleared of phosphates by precipitation with basic or neutral lead acetate.

Quantitative Determination.—Uranium nitrate method.

Phosphoric acid as a diacid salt is precipitated by uranium nitrate, and if cochineal be used as indicator the first excess of the uranium salt will give with it a green compound which serves as the end reaction. Uranium nitrate is preferable to the acetate, since its solutions are more stable, but even the nitrate is none too stable, and should be frequently restandardized. Since free nitric acid is liberated in the reaction, and this will dissolve a certain amount of uranium phosphate, sodium acetate is added in excess; and that all the phosphoric acid may be present as a diacid salt, acetic acid as well. The boiling urine should be titrated, since the end reaction is quicker and sharper, giving a more decided green.

Neubauer recommends that for greater accuracy the urine be precipitated with magnesium mixture and the precipitate washed on a small filter with dilute ammonia (water, 3 vols., 10 per cent. NH_4OH, 1 vol.). The precipitate is then dissolved in acetic acid, diluted to 50 cc. with water, and the titration continued as with the urine. The results obtained are somewhat lower.

Albumin and sugar may be present. The titer changes with the volume of reagent used. For instance, if 20 cc. are used, 1 cc. will indicate 4.98 mg. P_2O_5; 21 cc., 5 mg.; 40 cc., 5.14 mg. Hence the uranium nitrate fluid should be standardized against a phosphoric acid solution of about the concentration of normal urine.

The fluids necessary are, 1, a phosphate solution 50 cc. of which contain 0.1 gm. of P_2O_5. This is so difficult to prepare that we recommend that it be purchased from those chemists who make a specialty of such work. This is the standard solution.

2. A solution containing 100 gms. NaAc and 30 gms. acetic acid in 1 litre of water. Five cc. of this fluid added to 50 cc. of urine will keep all the phosphates in the diacid condition and prevent the presence of free nitric acid.

3. An alcohol cochineal extract; the ground cochineal insects digested in 25 per cent. alcohol.

4. Uranium nitrate solution, 1 litre of which contains 35.461 gms. of $UO_2(NO_3)_26H_2O$. This solution is standardized against solution 1. Three gms. of NaAc are added, since this salt always contains some free nitric acid. One cc. of this solution will indicate 5 mg. P_2O_5, hence 20 cc. should give the end reaction with exactly 50 cc. of solution 1.

To 50 cc. of the standard phosphate solution are added 5 cc. of solution 2, then a few drops of the cochineal tincture. The amount of indicator added is of moment, and a rather strong solution is desirable. This in an Erlenmeyer flask is brought to the boiling point. The uranium solution is then added to the boiling fluid in small amounts, shaking constantly. After each addition the precipitate is allowed to settle somewhat and the bottom of the flask studied for the first trace of green precipitate, the end reaction, which will first settle here. Having determined how much of this solution will exactly precipitate the phosphoric acid of 50 cc. of solution No. 1 it is then diluted to approximately the proper amount and then again for a final exact correction. Twenty cc. of this solution indicate 0.1 gm. of P_2O_5.

For the estimation of phosphoric acid in the urine 50 cc. of urine are treated in exactly the above manner. If very accurate results are desired a table of corrections for the change in titer necessary for the volume used should be at hand to make the necessary changes. If the urine be colored or jaundiced, the end reaction will not be sharp, and it should be acidified with hydrochloric acid or nitric acid and decolorized with $KMnO_4$. The urine should then again be neutralized. During the titration the flask should be kept on the water-bath or over the free flame to keep the fluid almost at the boiling point. Between each addition the precipitate should be allowed to settle that the first trace of green may be seen; the longer it is allowed to settle the sharper the end reaction.

Instead of the cochineal a 10 per cent. solution of potassium ferrocyanide solution may be used. In this case, after each addition of uranium nitrate one drop of the hot solution is brought into contact on a porcelain plate with one drop of this reagent. The end reaction is a brown precipitate. It should be remembered that this is not the same end reaction obtained by cochineal, but one considerably later, hence in using the fluids it is essential that one know with what indicator it was standardized.

The determination is very satisfactory, and a class all using the same urine get very close results.

Determination of the Acidity of the Urine.—The acidity of the urine is so much due to the presence of diacid phosphates that for many years the determination of these salts was the most accurate way of determining the acidity.

Freund's method is based on the fact that $BaCl_2$ will precipitate the monacid, but in the dilute urine not the diacid salt. If, therefore, the total phosphoric acid

and the monacid salt be determined, the difference will be diacid phosphate. The method is not exact; the monacid-salt figure will be 3 per cent. too great. For the determination the above solutions are used; also one of 100 gms. of $BaCl_2 2H_2O$ to 1 litre of water. The total phosphoric acid in 50 cc. of urine is determined. To 75 cc. of urine the barium chloride solution is added till the whole is 90 cc. The fluid is then shaken well and filtered until clear. This may mean repeated attempts; 60 cc. of the filtrate (equalling 50 cc. of urine) are then used, the phosphoric acid determined and subtracted from the total. From the monacid phosphate is subtracted 3 per cent., which is added to the diacid. The acidity of the urine is expressed in terms of the diacid salts.

Sulphates.—Sulphur is present in the urine in three forms,—(*a*) preformed or neutral sulphates; (*b*) ethereal or conjugated sulphates, that is, sulphuric acid combined with aromatic alcohols, indoxyl, skatoxyl, cresol, phenol, *et al.;* (*c*) neutral, unoxidized, or organic sulphur. Weighed as SO_3, $a + b$ amount to from 1.5 to 3 gms., or an average of 2.5 gms. of H_2SO_4 in twenty-four hours, in the case of a normal person on a mixed diet. As a rule, the ethereal sulphates are about one-tenth of the total sulphates. Since practically all of the sulphuric acid is a product of proteid metabolism, the output should be parallel to that of nitrogen, and the ratio between them is quite constantly 5 : 1 to 5 (100 : 19.1 to 20.4, Folin). But this is not exact, since the sulphur contained in proteids varies, and the amount of sulphur excreted in the neutral form varies as well.

The total sulphate output depends especially upon proteid metabolism, being increased in all conditions increasing proteid oxidization; hence there is none in the urine of the fœtus. It is especially dependent on a meat diet. It is increased by exercise, providing this increases the nitrogen output as well. It is increased in fevers, since in this condition there is an increased proteid catabolism. The increase is especially marked in acute inflammatory disease of the brain and cord, and in acute articular rheumatism. It is increased after protoplasmic poisons. It is diminished during convalescence from an acute fever and in practically all chronic diseases. The amount of total sulphates has very little clinical value.

The **ethereal sulphates** are of considerable interest. While their output is subject to great and inexplicable variations, they may be considered as an accurate index of the amount of absorption of the products of intestinal decomposition which can pair with the acid. They are independent in great degree of the neutral sulphates, and with the total sulphate their ratio varies so considerably that normal limits cannot be stated, hence it is their absolute amount which is of more value than their relative.

This amount varies in the first place with the food. They are increased in the urine of a dog fed on foul meat; are diminished during hunger and long fasting; they are diminished on a milk diet, in which case it is supposed that casein inhibits the bacteria of decomposition.

Intestinal decomposition increases them relatively and absolutely. This increase is diminished much by calomel and similar drugs. The decomposition of proteid furnishes phenol, cresol, indoxyl, skatoxyl, hydrochinin, pyrocatechin, and several other bodies; the most important, indol and phenol, explaining only about one-fifth, and a large percentage is still unidentified. They are increased by the ingestion of aromatic bodies, and particularly of carbolic acid. There is almost none in the urine of newborn. The output varies to a great extent with the hydrochloric acid of the gastric juice and the sodium chloride of the food. After hydrochloric acid medication they are diminished, and are increased by alkaline drugs.

Pathologically, they are increased in chronic intestinal catarrh and diminished in acute. They are increased sometimes in constipation, sometimes there is no change. In a recent interesting case of "black urine" (see page 94), from a case of extreme constipation, the total sulphuric acid (as SO_3) was only 0.147 gm. per 100 cc. of urine, and of this, 57 per cent. was ethereal sulphate; the following day the urine was of normal color, total SO_3, 0.086 gm. per 100 cc., and 50 per cent. of this ethereal sulphate. They are increased in those cases with defective absorption from the intestine, as in typhoid fever, intestinal tuberculosis, and peritonitis. They are increased in cholera, but during the stage of reaction there may be little or none present. In atrophic liver cirrhosis and carcinoma of the liver the increase is attributed to the accompanying intestinal catarrh. They are also increased if decomposition occurs in other parts of the body than the intestine. It is of interest that in gastric disease, even with much stagnation and fermentation, they are little affected.

The **unoxidized sulphur** is supposed by some to vary with the amount and quality of the food; by others to have relation not to food but to tissue destruction, to be increased by muscular work, the lack of oxygen, and the ingestion of various sulphur compounds, including the flower of sulphur, sulphonal, methylmerkaptan and ethyl sulphide.

This neutral sulphur, which amounts to from 14 to 25 per cent. of the total sulphur, is present in two forms: about 20 per cent. is the easily oxidizable, which is oxidized by bromine or chlorine (bromine is better, since chlorine attacks also the taurin derivatives), and the difficultly oxidizable. For the total the residue must be fused with KNO_3, since fuming HNO_3 does not oxidize all of the neutral sulphur. In cystinuria HCl plus KNO_3 will oxidize only from 30 to 40 per cent.

In jaundice from 24 to 60 per cent. of the sulphur is neutral, and of this there is an increase of about four to five times the normal proportion of the difficultly oxidizable form. In pneumonia the increase is of the easily oxidizable, and in liver disease the opposite. In cystinuria even 45.7 per cent. is neutral sulphur (in one of our cases 32 per cent.).

Edsall[39] carefully studied the easily split (by alkali) sulphur in a series of cases. His results were negative. He decided that cystinuria is the only disease with an increase in this sulphur fraction, and that the relative proportion of these two fractions has no clinical value.

Petry studied the question in dogs on a known diet, and found a quite constant amount (5.5 per cent.) of the total sulphur to be the easily split. This amount could not at all be influenced by diet.

Recent work by several (*e.g.*, Benedikt)[40] has emphasized the independence between the excretion of neutral and total sulphur, the former remaining almost constant whatever the diet. It is suggested that the neutral sulphur arises in the catabolism of particular proteids.

Detection and Approximate Estimation.—If in a test-tube holding over 25 cc. the urine is mixed with about one-third its volume of an acid barium chloride solution ($BaCl_2$, 4; HCl, 1; H_2O, 16 parts), a precipitate of the barium sulphate is formed. If this be a milky turbidity, the sulphates are normal; if creamy, increased; if merely a translucency, diminished. If allowed to settle from eighteen to twenty-four hours, and the precipitate fills one-half the concavity of the tube, they are normal. The above are the neutral sulphates. If this be filtered, and to the filtrate hydrochloric acid be added and the whole warmed, the ethereal sulphates are split and precipitated.

Quantitative Determination of Total Sulphuric Acid.—In the gravimetric method the barium salt is weighed. The ethereal sulphates must first be broken up by heating the urine with HCl. The precipitation must occur in hot solution and in the presence of free acid. The best concentration for filtration is a solution of not more than 0.1 per cent. $BaSO_4$. The chief difficulty in the whole process is to rid the precipitate of the barium nitrate, which it holds very fast.

Twenty-five or 50 cc. of urine are filtered, diluted from two to three times and from 5 to 10 cc. of hydrochloric acid per 100 cc. of fluid added. It is then heated to the boiling point for about fifteen minutes. Barium chloride is then added in slight excess. Huppert recommends that the beaker remain on the water-bath for several hours until the supernatant fluid is perfectly clear. It is then allowed to stand cold for twenty-four hours, that the salt held in solution by the hydrochloric acid may be completely precipitated, for $BaSO_4$, so insoluble in water, is somewhat soluble in HCl. The clear fluid is then decanted through a small ashless filter, the precipitate mixed with boiling water, and again allowed to settle until clear. This decanting is continued until the filtrate gives no clouding with silver nitrate. The precipitate is then brought on the paper, washed with hot water, hot alcohol to remove resinous matter, and then with ether. It is warmed, and dried to

[39] Univ. of Penn. Med. Bull., 1892, iii. p. 87.
[40] Zeitschr. f. klin. Med., 1899, vol. xxxvi. p. 281.

100° C. The precipitate is then separated from the filter onto a piece of glazed paper, the filter paper burned in a platinum spiral, and the ash added to the precipitate. This is then put into a platinum crucible and well burned. The crucible is then allowed to cool, a drop of sulphuric acid added, and again brought to a red heat. It is then cooled and weighed; again a drop of acid added, and the above continued until at constant weight. One hundred parts of $BaSO_4$ equal 34.28 parts of SO_3, 41.13 of SO_4, 41.99, H_2SO_4.

The hydrochloric acid, it is said, should be distilled, since it may contain a little sulphuric acid. The crucible should be heated by an alcohol flame, since illuminating gas may contribute to the sulphur in the crucible. Huppert recommends that, to avoid the loss of a certain amount of the $BaSO_4$ by its solubility in HCl, the HCl first be added, the urine then heated, then diluted with water and precipitated by the $BaCl_2$. In this way less hydrochloric acid is used.

The above method is long and tedious. The way recommended by Dr. Jones we have found much more satisfactory. The precipitation occurs in an Erlenmeyer flask covered by a watch-crystal; the fluid is then boiled vigorously over a free flame for about half an hour. The precipitate is then brought at once onto the filter paper, since there is little danger of its passing through. The S. & S. blue ribbon filter paper should be used.

Ethereal Sulphates.—These have been separated into those easily split and those difficultly split, according to whether heat is necessary for the cleavage by hydrochloric acid. They are not split by acetic acid.

The former are decomposed by HCl on standing in the cold for twenty-four hours. Such are compounds of indol,, skatol, etc. Compounds of phenol, kresol, *et al.*, are decomposed on the water-bath only.

Baumann recommended that to 50 cc. of urine be added acetic acid, and then an equal amount of water, and $BaCl_2$ in excess. This is then warmed over the water-bath from a half to three-quarters of an hour or until clear. It is filtered, washed, and the filtrate treated as for the total. The final weight will be that of the ethereal sulphate. To save time, since this precipitate passes easily through the filter, it is well to bring the mixture into a measuring cylinder and dilute to an easy volume, and then decant through a filter a given amount of the clear supernatant fluid.

Salkowski's method is much easier to apply. To 50 to 100 cc. of urine are added an equal amount of Ba mixture (1 volume cool saturated $BaCl_2$ plus 2 volumes cool saturated $Ba(OH)_2$). This is then filtered through a dry filter into a dry beaker. Fifty or 100 cc. of filtrate (equalling 25 or 50 cc. of the urine) are used. Hydrochloric acid is added until strongly acid (15 cc. to 100 of the filtrate)

and it is heated nearly to the boiling point. The further steps are the same as for the total sulphur. Kossel[41] has shown the possibility of certain errors in this method.

Neutral Sulphur.—Neutral sulphur may be determined in the filtrate of urine used for the total sulphuric acid, but it is much better to determine the total sulphur, and from this subtract the total sulphuric acid.

Total Sulphur.—Fifty cc. of normal urine (or 25 cc. of urine containing cystin) are evaporated to dryness. To the residue in a silver crucible is added a mixture of four parts potassium nitrate and 1 part of sodium carbonate (both sulphur free), and the whole burned until white. The residue is dissolved in water and poured into a porcelain dish, and the crucible well washed. It is then evaporated at least three times, adding hydrochloric acid each time to get rid of the nitric acid, all of which must be removed since nitrates are carried down in the precipitate and cannot be washed out. The residue is then dissolved in water, and allowed to stand, to see if any AgCl separates; if it does, filter. It is better to evaporate the urine almost to dryness before the addition of oxidizing mixtures, since the evaporation occurs more rapidly. Certain makes of porcelain crucibles can be used instead of silver, but the majority will break.

A method theoretically much better is Asbóth's modification of the Höhnel-Gläser method. In a nickel crucible are mixed about 1 gm. of the dry residue of the evaporated urine, 7.5 gms. of soda, and 10 gms. of sodium peroxide. The mixture is then heated over an alcohol lamp, the flame of which must not touch the crucible until perfect fusion. It is then heated to a thin fluid. This is allowed to cool, is dissolved in water, acidified with HCl containing some bromine, boiled till all the bromine is driven off, filtered into a beaker, and precipitated hot with a hot solution of $BaCl_2$. This method is advantageous, since no nitric acid is used. On the other hand, sodium peroxide is a violent explosive, hence the mixture with soda.

The oxidization is also done by diluting the urine with an equal amount of hydrochloric acid and then evaporating on a water-bath, from time to time adding a knife-point of $KClO_3$. When at dryness more HCl should be added and more $KClO_3$, until the residue on drying does not contain any brown particles. This method is easy, since no nitric acid is used. That, however, it gives the same result as the other methods we decidedly doubt, and are at a loss to explain the agreement which others have found.

In a series of metabolism experiments we used always both this and the fusion with KNO_3 and soda, and were at a loss to explain their lack of agreement. In collating all our results we found, to our surprise, that the difference was almost a constant, the HCl-$KClO_3$ method giving about 80 to 85 per cent. of that by the

[41] Zeitschr. f. physiol. Chem., vii. p. 296.

KNO_3-soda method, and this true, although the totals differed considerably. This may throw some light on the sulphur distribution. (See below.)

Easily Oxidized Sulphur.—Jerome's method. To 50 cc. of urine are added 40 gms. of $KClO_3$ in a flask, and this warmed on a sand-bath with the addition from time to time of small portions of 100 cc. of HCl. The solution is evaporated on a porcelain dish to dryness and is then evaporated with 100 cc. of HCl three times. It is then filtered and the process continued as for sulphuric acid. It will be noted that this old method for easily oxidizable sulphur is that which recent workers have used for total sulphur. (See above.)

Thiosulphuric Acid, $H_2S_2O_3$.—Normally there is in the urine of man none, or not over 10 mg. in 1 litre. It has been found, however, in some cases, as, for instance, in typhoid fever.

Hydrogen Sulphide, H_2S.—This seldom occurs in fresh urine. It has, however, been found, and by it have been explained cases of autointoxication. It was found in one case of long-standing eclamptic coma. It soon appears, however, in a urine on standing, and can be produced in any normal urine by warming it with a mineral acid. It does not occur in disease with decomposition in the intestine, nor after the ingestion of alkaline sulphides, nor after sulphur baths. While it is doubtful whether it can reach the bladder from the rectum, it can arise in the bladder from fermentation processes. It develops in the urine from decomposition due to organisms, at least eight of which have been described as specific. Müller considers that it is derived from the unoxidized sulphur. It may be detected in the fresh urine by the odor, or by suspending in the mouth of the flask a strip of paper moistened with sugar of lead solution plus one drop of NaOH. Air should then be aspirated through the urine. The paper will be blackened.

Sulphocyanic Acid, HSCN.—This acid occurs normally in the urine of man and the animals which excrete nitrogen as urea, in amounts equalling about one-third of the neutral sulphur. The amount seems constant in an individual, but varies in different persons. It is increased by the inhalation of CS_2 even fifty times.

To 100 cc. of urine are added HNO_3 and $AgNO_3$. The precipitate is filtered, washed, suspended in water, decomposed with H_2S, and the filtrate distilled. The distillate is tested with Fe_2Cl_6, giving an intense blue fluid (Berlin blue) not modified by HCl.

Carbonates.—Carbonic acid is present in the urine, both free, which may be removed by a vacuum, and bound, in which case acid must be added to free it. Of the free there are about 180 cc. of CO_2; of the bound, from 2 to 10 cc. The carbonic acid is increased by a diet rich in organic acids which are oxidized to carbonates, hence it is present in great abundance in the alkaline urine of herbivora. In urine there is little in solution, the most being united with a base, and its solubility depends on the relative amount of CO_2 and MH_2PO_4; if some of the CO_2 is removed by a vacuum, the acid phosphate at once sets more CO_2 free until none is left. The carbonic acid may be determined by drawing a stream of air through the urine and then through clear baryta water, which it clouds at once. If, then, the urine be acidified, the bound CO_2 will be free. The amount of CO_2 is greatly increased as the urine begins to decompose, a point quite disturbing in all quantitative work. In some cases of nephritis, especially if on an alkaline treatment, the urine is alkaline when voided, and contains so much carbonate that quantitative work is impossible.

Silicic Acid is present in traces as silicates. Its source is the food.

Nitric Acid is present in all normal urines as nitrates. This also is from the food and especially certain vegetables.

Nitrous Acid is often found as nitrites, but is reduced from the nitrates by the bacteria.

Calcium and Magnesium.—These alkaline earths are excreted as phosphates to the amount of about 1 gm. per day; calcium, weighed as CaO, about 0.12 to 0.25 gm., and MgO from 0.18 to 0.28 gm. per day. The calcium excretion, even of that injected subcutaneously, is chiefly through the intestines, and from but 4 to 29 per cent. through the urine. On this account the calcium in the urine is no index of the amount absorbed from the intestine. In the urine its output is parallel to that of the ammonia, and it seems to bear some relation to the excretion of acids. In this connection it is of interest that most is excreted in the morning, at which time the urine is most acid. Its chief source is the food. During starvation periods calcium is increased relatively and absolutely, of interest since a slight acidosis exists also then; the source of this calcium is assumed to be the bones. It can be decreased by alkaline treatment. On a vegetable diet there is only a trace of calcium in the urine. It seems increased by exercise.

The factors influencing the output of calcium are little understood, and yet much would indicate that the calcium bears some relation to the condition known as acidosis (see page 193) There is no increase in tuberculosis, none in rickets. In chronic diseases the increase can be explained by inanition. In diabetes an interesting behavior of this metal was demonstrated by Gerhardt and Schlesinger, who by very careful metabolism work confirmed the previous findings that the output is in diabetes increased even two to four times the normal amount; that the output is parallel to that of ammonia and can be diminished by alkaline treatment; and that in those cases with acidosis it is especially increased; the normal ratio between the intestinal and the urinary output is reversed in favor of the latter, while there seems a certain amount of retention of magnesium in the body. In the case of arteriosclerosis a retention of calcium has been demonstrated.

The relation of this metal to phosphaturia is interesting, since that symptom-complex would seem to be due more to an increase of calcium with a diminution in the phosphoric acid than to an increase of the latter.

Quantitative Determination of Calcium.—Two hundred cc. of filtered urine are made alkaline with ammonia until there is a distinct precipitate. This is then dissolved in the smallest amount of hydrochloric acid with the addition of some NaAc. Ammonium oxalate is then added in excess and the fluid allowed to stand covered on a water-bath for twelve hours. The precipitation of calcium phosphate should be avoided, which occurs if no ammonium be added or if the urine be foul. The supernatant fluid is then decanted through a small ashless filter, the precipitate washed Cl free by decantation with hot water, and then finally brought on the paper. The precipitate of CaO is very fine and apt to pass through the paper,

hence is washed as much as possible by decantation. The wash-water may be saved for magnesium determination. During this process bacterial fermentation should be prevented by thymol or carbolic acid. The dry filter paper is then put in a platinum dish, burned moderately for a long time, then at a dull red, till the mass on cooling is perfectly white. It now contains some oxide. It is moistened with a concentrated solution of ammonium carbonate, slowly dried, and very gently ignited. The treatment with ammonium carbonate is repeated till of constant weight as calcium carbonate. One part of $CaCO_3$ equals 0.40 parts of Ca. Or the mass may be burned white with a blast-flame. The crucible is then cooled and weighed and the blast repeated until the weight is constant. The precipitate is now CaO, 1 part equalling 1.845 of calcium phosphate. Or the precipitate is burned white, then concentrated ammonium sulphate added, again burned, and this repeated until there is no increase in weight. One part of this calcium sulphate equals 0.41176 part of CaO.

Quantitative Determination of Magnesium.—For this the filtrate and wash-water of the above determination may be used. One-third volume of 10 per cent. NH_4OH is added (sp. gr. 0.96), which will precipitate all of the Mg as NH_4MgPO_4. This is allowed to settle well, collected on an ashless filter, washed with water plus one-third volume of ammonia, thoroughly dried, shaken into a platinum crucible, the paper burned in a platinum spiral and its ash added to the crucible, and the whole then fused. Since there is some uric acid in the precipitate it will hardly burn white. It should therefore be cooled, a small piece of NH_4NO_3 plus a few drops of water added, this warmed slowly, and then finally burned. The result is $Mg_2P_2O_7$. One hundred parts of this equal 36.208 parts of MgO.

It is a saving of time to treat 200 cc. of the original urine in this way. The result is calcium and magnesium. The Ca is determined in a second portion, and the difference will be the Mg.

Sodium and Potassium are present, of the former 4.2 to 7.4 gms. as Na_2O in twenty-four hours, and of the K_2O from 2.3 to 3.9 gms., the usual relation between them being 5 : 3.

Their amount depends on the food. In hunger the potassium may exceed the sodium, also in fever, but after the crisis the sodium will predominate.

Severe exercise and vegetable diet increase the potassium.

Iron.—A trace of iron is always present in the urine in organic combination. The figures given of the amount found vary widely, since all methods have many sources of error, in many cases the iron of the reagent used being greater in amount than that to be determined. The figures given vary from 1 to 10 mg. in twenty-four hours. This is increased in fever, the amount varying as the height and duration of the elevation of temperature. Large amounts have been found in malaria (even 16 mg. a day), pernicious anæmia, and alcoholics. This question has recently been studied by Neumann, who considers that his method of ashing the urine allows a very accurate estimation of the iron. Neumann and Mayer [42] found that the output of a normal person varied from 0.93 to 1.139 mg., an average of 0.983. In pathological urines they found it increased, especially in alcoholics. Their finding concerning diabetes is very interesting, since the iron output was parallel to that of the sugar; the ratio

[42] Zeitschr. f. physiol. Chem., 1902, vol. xxxvii. p. 2.

being quite constantly 2.5 mg. per 100 gms. of sugar. They therefore suspect that both bodies have a common source.

Lead.—Considerable urine is evaporated to dryness, and 50 cc. of fuming HNO_3 added; after the reaction subsides it is allowed to simmer over the free flame for half an hour, and then 25 cc. more acid added each fifteen minutes three times. The fluid is then evaporated to small volume, neutralized with NaOH, filtered, and the lead tested with H_2S, which will give a brown precipitate.

Arsenic may be tested by saturating the faintly acid urine with H_2S, allowing it to stand from twelve to twenty-four hours, filtering, washing, and treating the precipitate with bromine water, which will dissolve the arsenic sulphide. The solution is placed in a suitable flask, to which is added zinc and sulphuric acid, and the stream of hydrogen conducted into an acid $AgNO_3$ solution ($AgNO_3$, 0.1 to 0.2 gm.; HNO_3, 2 gms.; water, 10 cc.). If AsH_3 is generated, one gets a blackish-brown precipitate of metallic arsenic.

PIGMENTS OF THE URINE

The value of the ethereal compounds of sulphuric, glycuronic, and other acids has been overestimated, yet they have a certain clinical importance.

Indoxyl sulphate, the chief body, originates in the intestine as indol which is formed in the decomposition of proteid. Indol is absorbed from the intestine, and in the body is oxidized to indoxyl, conjugated with sulphuric acid, and excreted as an alkaline salt. In men its output is greater on a flesh than on a vegetable diet. In the case of fasting persons it arises from the decomposition of the intestinal secretions. There is none in the urine of the new-born or until the child is fed cow's milk. In adults a certain amount is always present, from 5 to 25 mg. in twenty-four hours on a mixed diet; hence only a great increase is of value, and this may reach from 50 to 150 mg.

In general, it is increased by the rapid decomposition of albumin either in the intestine or elsewhere in the body. The increase in cases with limited peristalsis—*e.g.*, peritonitis and ileus—is of importance only to indicate the location of the obstruction or of the paralysis of the bowel. If of the small intestine, there is a great and rapid increase; of the colon, none or late. Its formation seems to depend upon the presence of trypsin, which before it reaches the colon has been either destroyed or reabsorbed, hence there is most indol formed in obstruction of the small intestine. It cannot be used to distinguish peritonitis from a twist of the bowel. In one very interesting case of syphilitic stricture of the ileum the obstruction, which occurred frequently, due evidently to the accumulation of fecal matter above

the constriction, could be foretold by the increase of this body, which reached a high point and then, as the food gradually passed on, cleared up much. It is much increased in intussusception, new growths, and twists of the small intestine. It is increased by intestinal putrefaction, such as occurs in diarrhœa, especially the cholera infantum of children, in typhoid fever, dilated stomach, and some cases of nephritis. In these conditions brisk purging diminishes the output greatly.

It is increased by decomposition of albumin elsewhere in the body, as, for instance, gangrene of the lung tissue, gangrene of a pleural exudate, empyema, putrid bronchitis in which case it may be very large in amount, advanced pulmonary tuberculosis, and advanced intestinal tuberculosis. Coriat considers its increase one element of the symptom-complex of akinetic mental conditions and its diminution of the hyperkinetic states. He considers this not due to any intestinal condition nor to the diet. In certain cases of chronic constipation there is much present. One such case, a colleague of mine, furnished my classes for several years specimens of urine containing much. This person enjoyed the best of health. He gives a history of some severe abdominal condition when ten years of age, since which time he has been troubled with constipation.

His urine on one day was as follows:

Total amount, 1770 cc., clear yellow color. On boiling, it becomes dark brownish-red, almost black, with a dark magenta foam.

Total SO_3, 1.59 gms.; ethereal sulphates, only 14 per cent.! Total sulphur, 1.82 gms. (as SO_3) in twenty-four hours.

The urine gives a splendid indigo-blue test, not the Rosenbach test.

It will be seen that despite the color on boiling and the good indoxyl test, the ethereal sulphates were not increased.

The output is diminished by closure of the pancreatic duct, but this closure cannot be diagnosed from the absence of indoxyl sulphate unless other factors which would favor its formation are present. A former idea was that it is increased in conditions of inanition and tuberculosis, and there is a long list of diseases in which it has been found in abundance; various intestinal troubles, cancers of the liver, stomach, or uterus, and lead colic. In cases of peritonitis or of appendicitis with abscess, an increase of indican is an unfavorable sign; its decrease, a favorable one. It is increased when the HCl of the gastric juice is diminished. It seems to bear some relation to the albumin output, and the insurance companies are beginning to consider it an early feature of nephritis.

Phenol is almost always increased with it; the reverse, however, is not true.

The urine when voided is, as a rule, normal in color. In certain cases the oxidation occurs within the body and the urine is green

when voided (the blue of the indigo plus the yellow of the urine). Such cases are described by Sahli, MacPhedran and Goldie, and others, but are excessively rare, and methylene blue is always to be excluded (see page 94).

Indigo calculi have been found.

The demonstration of indoxyl depends on its oxidation to indigo-blue, and the index of amount is the amount of indigo thus formed.

$$2C_8H_6NKSO_4 + O_2 = 2C_8H_5NO + 2KHSO_4$$

This reaction occurs if an oxidizing agent be added; also when the urine decomposes, in which case it is present as a copper-red scum with a metallic glistening. Rarely, however, is there sufficient to be seen grossly.

Indigo-blue is a dark blue powder, insoluble in water, slightly so in chloroform, easily soluble in hot aniline. It is insoluble in alcohol and ether. It should be collected on an asbestos filter, washed with water, then with alcohol (to separate the indigo-red), and then dried.

Tests: It may be sublimed at 300° C., giving off a purple-red vapor which cools in prismatic crystals of a copper-red metallic color, deep blue on transmitted light. If indigo-blue be mixed with hot alcohol, very strong NaOH, and some glucose, the whole filling a closed flask, indigo-white is formed. If then exposed to the air, indigo-blue will recrystallize out.

TESTS OF INDOXYL SULPHATE. *Jaffé's Test.*—A test-tube is half filled with urine, another of the same size with the same amount of concentrated HCl. On the edge of this latter test-tube is placed the smallest drop of fresh concentrated $Ca(ClO)_2$. The HCl is then poured quickly into the urine, carrying with it this drop as it flows over the edge. The fluids are mixed rapidly by inverting, not by shaking the tube. One cubic centimetre or so of chloroform is then added, which will extract the indigo as it is formed. If necessary then another drop or two of the $Ca(ClO)_2$ solution may be added. The test may be performed as a contact test. Hammersten advises that to 20 cc. of urine 2 or 3 cc. of chloroform be added, and an equal amount of hydrochloric acid; then at once $Ca(ClO)_2$ drop by drop, reversing the tube several times after each addition. The difficulty with this test is that a slight excess of the hypochlorite will destroy the indigo, giving yellow isatin. Albumin must be first removed by boiling and filtering.

$Ca(ClO)_2$ is a difficult substance to obtain pure, since it deteriorates so rapidly that the manufacturing chemists refuse to import it. A pure salt, however, is unnecessary, the ordinary cheap bleaching powder or "chloride of lime" being satisfactory. (Chloride of lime, it should be remembered, is not calcium chloride, but a mixture of calcium hydroxide, calcium chloride, and calcium hypochlorite).

Obermayer's Test.—The urine is first precipitated with about one-fifth volume of 20 per cent. PbAc, avoiding an excess, then filtered. Disturbing substances are thus removed. An equal amount of fuming

hydrochloric acid containing Fe_2Cl_6 is then added (4 parts of Fe_2Cl_6 in 1 litre of HCl). In a few minutes the reaction is apparent.

If potassium iodide is in the urine, a violet color is obtained. With thymol the urine becomes bluish-green.

Aman's reaction with $Na_2S_2O_7$ has been found of doubtful value.

The test may also be performed by adding 30 drops of urine to 15 cc. of HCl, plus 1 or 2 drops of HNO_3. The mixture is stirred at once, and amethyst color results, which reaches a maximum in from five to thirty minutes.

Since nitric acid gives the test, indol may disturb a bile test. In case the urine looks grossly as if indigo were present (the color of such a urine will be blackish-green or bluish), the indigo may be extracted with acidulated chloroform.

Indigo will crystallize out in needles or plates if evaporated from chloroform.

Quantitative Determinations.—These are in general unsatisfactory, the gross estimate by the color, using the Obermayer reagent, being usually sufficient. This urine may be repeatedly extracted, the extracts evaporated in a weighed beaker. The indigo-red may in this case be removed by washing with alcohol. It is dried at 105° to 110° C. and weighed.

Ellinger[43] found that but 85 per cent. of the theoretical amount was in this way obtained (evidently isatin is formed), and that neither the concentration nor the excess of reagent was of moment if one quickly extracted the indigo-blue. The urine is, if necessary, made slightly acid with acetic acid, precipitated with one-tenth volume of PbAc and, if concentrated, diluted one-half. To a measured portion of the filtrate is then added an equal volume of Obermayer's reagent, and shaken out several times with chloroform until this is no longer colored. The amount of filtrate chosen should be such that three to four extractions with 30 cc. of chloroform, for two minutes each time, is enough. The filtered extract is distilled, the extract dried for five minutes, then washed out two to three times with hot water (to remove isatin), dissolved in 10 cc. of concentrated H_2SO_4, this solution diluted to 100 cc. with water, and titrated with a dilute $KMnO_4$ solution (5 cc. of a 0.3 per cent. solution diluted to 200 cc.) which has been standardized with pure indigo-blue. About 87 per cent. of the correct amount is found, hence the result may be increased by one-sixth. A double determination requires about one and a half hours.

Strauss[44] also uses Obermayer's solution and extracts with chloroform. He uses a small separating funnel similar to that for lactic acid (see Fig. 63). The combined chloroform extracts are measured, 2 cc. removed and diluted till its color matches that of a standard tube of known content, and from this he reckons the total amount.

Bouma's method[45] has received severe criticism.

Coriat proposes[46] a graduated separating funnel, in which the $Ca(ClO)_2$ test is made and the chloroform extract compared with a standard color.

Skatoxyl-sulphate.—Skatol is also formed in the intestine, the result of the bacterial decomposition of albumin, and is absorbed. By analogy we may suppose it to be oxidized to skatoxyl, conjugated with sulphuric acid, and eliminated by the urine. As a matter of fact, however, the skatoxyl sulphuric acid has seldom, if ever, been actually demonstrated in the urine, and the colors which would indicate it may be as well due to other red pigments.

[43] Zeitschr. f. physiol. Chem., vol. xxxviii. p. 178.
[44] Deutsche med. Wochenschr., April 17, 1902.
[45] Zeitschr. f. physiol. Chem., 1901, vol. xxxii. p. 82.
[46] Am. Jour. Med. Sci., April, 1902.

Stokvis[47] has given a method for the separation of the chromogens of these blue and red pigments. The urine is saturated with $(NH_4)_2SO_4$, allowed to stand till all pigments are precipitated, filtered, the filtrate evaporated on the water-bath, and the fluid decanted from the crystals of $(NH_4)_2SO_4$. It is then acidified with a few drops of acetic acid, and shaken out with an equal volume of acetic ether, which takes up both chromogens to a yellow solution. To the acetic ether is now added water several times to separate out the chromogen of indigo-blue. It is then neutralized with dilute KOH and shaken out, the dilute KOH separating the chromogen of skatol-red, which can be tested with Obermayer's solution.

The red or violet color produced by adding an oxidizing agent with a strong acid to the urine is usually attributed to this body. With Fe_2Cl_6 it does give a violet color, with concentrated HNO_3 a cherry-red color, with concentrated HCl it is decomposed with a red precipitate. In Jaffé's test the urine is dark red or violet. On standing in the air the urine becomes darker from above downward,—of a red, violet, or even black color. As has already been mentioned, these colors are not conclusive. Rosin denies that it has ever been found, and thinks all of these tests could be explained by indigo-red. Its demonstration would necessitate the reduction of the pigment by zinc-dust with skatol as the product.

Indigo-red.—Other names for this pigment are many, urorubin and urorhodin being among them. This body is always formed with indigo-blue, especially by Jaffé's test with the warm urine. They are isomeres and arise from the same mother substance (indoxyl sulphate). It is also formed in decomposing urine and may form a sediment. Urorosein is formed at the same time.

Indigo-red crystallizes in dark reddish-brown or chocolate-brown needles or plates. It sublimes with violet-red fumes at 295° to 310° C. It is insoluble in water, dilute acids, and alkalies. It gives a cherry-red solution with alcohol, ether, chloroform, and especially glacial acetic acid. From dilute alcohol solution it precipitates in crystals. From glacial acetic acid it is precipitated by soda or by water. It gives a characteristic absorption spectrum.

Reduction Test.—The alcohol solution is made alkaline with sodium carbonate, a little glucose added, and gently warmed. The solution is decolorized, but the color returns on shaking it in the air. This can be repeated as often as desired. If the pigment be boiled with caustic alkali, even dilute, the red is destroyed and various brown decomposition products formed.

This pigment is present in large amounts in certain urines which give Rosenbach's test, but it alone is not responsible for the Burgundy-red color. It is increased especially in intestinal troubles,—ileus, obstruction, cancer, etc. It is also present in large amounts in some urines which do not give a characteristic Rosenbach test, but these conditions are so various that they cannot be classified. It may also be found in traces in normal urine.

Demonstration.—Nitric acid is the best reagent, its addition giving a red color. Much is formed by Jaffé's test, especially if the urine be heated. When cold, the urine may be neutralized with soda, then shaken out with ether. The ether takes a fine red color and gives the absorption spectrum of this body. The ether extract may be evaporated in a watch-glass to obtain crystals.

Indigo-red is present in certain freshly voided urines as in cases of pyelocystitis. It has also been found in concretions.

[47] Centr. f. inn. Med., 1902, No. 28.

Among other red pigments of the urine is UROROSEIN. This is characterized by its easy solubility in amyl alcohol, its insolubility in chloroform, ether, and benzol. Ammonia and alkaline carbonates decolorize it at once; acid restores the color. It may be recognized from its spectrum. It is very unstable and decomposes rapidly. It occurs in normal urine.

To demonstrate it one-tenth volume of HCl is added, and then the fluid is filtered. The red stain which remains on the filter is urorosein. Urorosein is produced by Jaffé's test, but is not extracted by the chloroform or ether. Other red pigments may be demonstrated in the urine, and after boiling it with acid various brown pigments with which it is tempting to work but not very profitable. These pigments, somewhat similar in appearance but different in their solubilities, will puzzle one considerably.

Paracresol and Phenolsulphuric Acid.—Phenol is present in the urine, from 17 to 51 mg. per twenty-four hours. The amount of these two bodies, however, of which the former usually exceeds in amount, varies in various conditions. It is increased with a vegetable diet. In ileus and peritonitis much phenol is present, also in diphtheria, scarlet fever, erysipelas; but little in typhoid fever, smallpox, and meningitis. They are formed from decomposition in any part of the body and when the intestinal decomposition is much increased. Their occurrence is thus much the same as indoxylsulphuric acid, the phenol increasing with this, but the reverse is not always the case.

Pyrocatechin (see page 93) is another body conjugated with sulphuric acid, and

Hydrochinon (see page 93) also, especially after carbolic acid poisoning.

Potassium Iodide is often found in the urine when making the tests for pigments. If HNO_3 be added, and then chloroform, the latter will take the pink color of iodine. Or powdered starch may be added after the HNO_3, and the starch iodine blue will be very distinct.

BILE PIGMENTS

The Clinical Occurrence of Bile Pigments in the Urine.—Bile pigment never occurs in the human urine normally, although it is a constituent of the normal urine of some animals. Its origin is the blood pigment, as may be seen by the fact that it is increased and the intermediate stages occur in the plasma, when there is increased breaking down of red blood-cells,—*e.g.*, in hæmoglobinæmia, after a blood poison. It is very similar to hæmatin, and is an isomer of hæmatoporphyrin. In cholæmia it is thought that the most of the bilirubin is reduced by the kidneys to the more diffusible urobilin.

The cases of jaundice have been divided into two groups,—the "hepatogenous," due to obstruction of bile passages, in which case bile

is present in the urine sooner or later, as in cases of catarrhal jaundice, jaundice due to calculus, cancer or cirrhosis of the liver; and the "hæmatogenous," formerly supposed to be due to destruction of the red blood-corpuscles by poisons, as phosphorus or the toxines of severe infections, in which cases bile may appear in the urine before the skin or conjunctivæ are stained. The previous idea was that in these latter cases the liver could not warehouse all of the free hæmoglobin, and hence some was excreted as bilirubin. More recently such jaundice has been doubted (Stadelmann), and all cases are supposed to be hepatogenous in origin; that is, the increased bile pigment is reabsorbed in the liver, since it is perhaps too viscid to flow well through the bile ducts, or there is too little pressure from behind, or perhaps there is closure of the smallest ducts from swelling of the cells. "Toxæmic jaundice" has been proposed as a better term.

The bile pigments, and their derivatives of interest in clinical chemistry, are bilirubin, biliverdin, bilifuscin, biliprasin, cholecyanin, and choletelin, all of which are products of bilirubin, and many of which are seen in the play of colors of the Gmelin test. The first two, bilirubin and biliverdin, are the only ones of much importance except in explaining the colors in tests. Bilirubin alone has been proved in fresh urine. Biliverdin often occurs, but, as a rule, only after the urine has stood for even a short time, in which case it is the effect of oxidization by bacterial action. These pigments are always present in the urine in cases of jaundice of much intensity. It should be remembered, however, that all of the pigment may be in the urate sediment.

Bilirubin, $C_{32}H_{36}N_4O_6$.—This is the pigment which occurs free in the bile of man. Its calcium salts occur in gall-stones, its crystals occur in old blood extravasations, the so-called hæmatoidin crystals supposed formerly to be a different substance but now generally admitted to be the same. It is probable that bilirubin can arise elsewhere than in the liver. It occurs in the fluid of certain cysts, especially of the breast and of the thyroid.

This pigment may be isolated from fluids containing other pigments by precipitating with the milk of lime in moderate amount, shaking well. CO_2 is led in at once to prevent the decomposition of methæmoglobin et al., and the mixture filtered. The precipitate is washed, and dissolved in alcohol; chloroform is then added, and then acetic acid, to separate out the calcium. This is filtered, the chloroform separated by adding water, and the chloroform extract is filtered through a dry paper and evaporated. The bilirubin of the residue is washed with a little alcohol and ether. The results by this method are always a little too low, since calcium does not precipitate all the pigment and some is later destroyed. The work must be done rapidly. Hæmatin, if present, would also be isolated, but hæmatin does not occur in the body, except perhaps in the stomach and intestinal contents.

The crystals are rhombs often with rounded edges, or needles, if pure of a beautiful brown-red color. It is perfectly insoluble in water,

soluble in alcohol and in chloroform, especially if hot, to solutions of a brownish-red color. It is precipitated unchanged from alcohol solution by acids, forms compounds with alkalies, these compounds being insoluble in chloroform but soluble in water; hence bilirubin may be washed from a chloroform solution by an alkali. In this it differs from lutein. It is precipitated by $BaSO_4$ and $(NH_4)_2SO_4$. The alkaline solution exposed to the air turns to the green biliverdin. In the alkaline urine, however, this is not always the case, since one product of the decomposition of urine is $(NH_4)_2S$, which changes biliverdin and bilicyanin to bilirubin, and the bilirubin itself may disappear from an alkaline urine, also from a urine preserved with chloroform. Bilirubin has no absorption spectrum.

Biliverdin, $C_{32}H_{36}N_4O_8$, occurs in the bile of many animals, but not of men. It occurs, however, in the intestine and vomitus, and in jaundiced urine after standing even for a short time. When pure it is an amorphous, greenish-black powder, insoluble in water, ether, or chloroform, but easily soluble in alcohol. Its compounds with the alkalies are soluble to a green or a brownish-green solution. It is soluble in concentrated acetic acid and in HCl. The alkaline solution has no absorption spectrum, but an alcoholic weakly acid solution shows one band. With Gmelin's test it gives the same color changes as bilirubin, from which it differs in its color, solubility in alcohol, and insolubility in chloroform. It may be reduced to bilirubin.

Hydrobilirubin, $C_{32}H_{46}N_2O_7$, is considered by some to be the same as urobilin, but this is denied by the majority. This occurs in the lower intestine as a reduced product of bilirubin by bacteria.

Bilifuscin is a body not yet isolated pure. That described ($C_{32}H_{40}N_4O_8$) is of an amorphous brown color, soluble in alcohol to a deep brown solution, and in alkali, ammonia, and dilute NaOH. It is insoluble in water and ether, and nearly soluble in chloroform. It is soluble in ether and chloroform if fatty acids be present. In the pure state it does not give Gmelin reaction; its spectrum is similar to that of biliprasin.

Biliprasin is commonly said to be a mixture of bilirubin and bilifuscin. By others it is said to be an intermediate stage between bilirubin and biliverdin. Others consider it identical with biliverdin. The formula given is $C_{32}H_{44}N_4O_{12}$. The alcoholic solution has no absorption spectrum, but there is of the alkaline solution. The color of its alcoholic alkaline solution is brown, the chief difference between this and biliverdin. It differs from bilifuscin, since if acid be added to this solution, the color changes to green. The Gmelin test is of no value in recognizing this pigment.

Of the above pigments, the spectrum analysis is unsatisfactory for their recognition. It is the products of their oxidation which are easily recognized by this method.

Cholecyanin.—This is an oxidized product of the bile pigments with nitric acid, PbO, or $KMnO_4$. It gives a characteristic spectrum, and may be further oxidized to choletelin. Cholecyanin is insoluble in H_2O, soluble in alkalies and strong acids. It may be reduced to bilirubin. The neutral or faintly acid solution is of a bluish-green or steel-blue color with a beautiful red fluorescence. The

alkaline solution is of a green color. The only way of recognizing it is by its beautiful spectrum.

Choletelin.—Choletelin may be produced by the oxidation of bilirubin with HNO_3. It is soluble in alcohol giving a ruby-red color. The dilute solution is a yellowish-red, and does not change in color with the change of reaction, as is the case of urobilin. It does not fluoresce. Chemically it is similar to urobilin, but its absorption spectrum differs, and with $ZnCl_2$ it gives no fluorescence. It is not precipitated by PbAc. In testing the spectrum the solution must be made acid with acetic acid, and the lines for urobilin must not be mistaken. The spectrum is the only means of recognition.

The reducible body of Stokvis is a by-product of the complete oxidation of bile pigment. It is a substance soluble in water, alcohol, alkali, and dilute acid, but not in ether or chloroform. It is not precipitated by PbAc, but is by PbAc and NH_4OH. It is characterized by the fact that if its alkaline solution be boiled with a reducing substance (*e.g.*, $(NH_4)_2S$), the solution becomes a beautiful rose-red with an absorption spectrum. This shaken with air, the rose red disappears, and it is restored to its original color with the disappearance of the spectrum.

The *color* of the urine does not depend alone on the amount of bilirubin present, for much may be present in a pale urine and little in a very dark. In general it varies from a dark yellow to brown or even greenish-black. The most characteristic feature is the yellow foam, since in a very dark non-jaundiced urine the foam produced by shaking is pure white unless much urobilin be present. The urine has a yellow sediment and stains the filter paper yellow. As a rule, in such urine there is also an excess of urobilin and indoxyl, hence when the bilirubin disappears the color of the urine still remains dark. It also contains the nucleo-albumin of the bile. Such urines are not adapted to Heller's albumin test since the oxidized pigment will confuse one.

Tests.—If there be much bile in the urine it may be shaken out with chloroform, the chloroform extract poured off, evaporated, the residue taken up again with chloroform, and evaporated in a watch-glass. Rhombic prisms of bilirubin are seen which are soluble in alkali, give the Gmelin test, and on exposure to the air become green.

Gmelin's Test.—The urine is superimposed in a test-tube on crude HNO_3 (sp. gr. at least 1.4). The urine is best added with a pipette and these fluids stratified as for the albumin test. The HNO_3 should be faintly yellow with HNO_2, not too much nor too little so. The yellow may be increased by adding a few pine shavings to the nitric acid or diminished by adding a little urea.

If bilirubin be present, strata of colors will be seen in the urine which from above downward are green, blue, violet, red, and, just above the HNO_3, yellow.

If too much HNO_2 be present, soon the whole is yellow. This test cannot be applied to a very dark urine or to a urine rich in indican. If the latter be present, the blue of indigo may with the yellow of the urine give a deceptive green. The ring has a black tone, and a fine precipitate can be seen. If in doubt the urine may be extracted with chloroform and both pigments tested for. A violet-red ring may be due to skatoxyl. Often only a green color is seen. This is necessary for

diagnosis, and most persons agree is sufficient. The violet-red, according to others, must also be present, else the test may be confused with that of lutein, which gives a blue or a bluish-green ring; but in the case of urine this pigment will not disturb. The violet-red is due to skatol and indoxyl. Biliverdin also gives this test, but the reaction occurs in a shorter time since the first oxidation step is already present. The test may fail if too much HNO_2 be in the nitric acid, since the green will not be seen.

Alcoholic solutions cannot be tested, since alcohol alone will give this test. If urines are shaken out with ether, the ether must be alcohol-free, and this is not always the case.

If urobilin be abundant, the bile test will be poor, but, as a rule, it does not disturb much. The urine should be diluted to a specific gravity of 1005, and the green bile test obtained. Some prefer always to dilute, since then only the green is seen.

Lutein of the serum does not disturb, but methæmoglobin may. An abundant albumin precipitate will obscure the test, and if the albumin be removed it will carry the bile down with it. This albumin precipitate should be dried and extracted with chloroform. A trace of albumin will not disturb the test, and if much bile be present will even improve it, since so much will be carried down with the precipitate, but if only a trace of bile be present and a trace of albumin, the latter must be precipitated, dried, and extracted.

The Gmelin test is said to indicate as little as 1 : 80,000.

After the ingestion of antipyrin it is said that the test is positive.

Rosenbach's test is the best modification of Gmelin's test, being the most sensitive. Much urine is filtered several times through a filter paper, which will retain the bile-stained elements of the sediment. The filter is then partially dried with dry filter paper and one drop of yellow HNO_3 dropped upon it. Rings will be seen which will present the above-mentioned play of colors, the external one being green. The urine should be somewhat acidified with HCl before filtering. If the paper be allowed to dry, it should be again moistened with water before the test. Instead of filter paper Dragendorff uses a porous porcelain plate.

A test considered by some very delicate is the following. A test-tube is filled full of urine, and 2 cc. of chloroform and 3 drops of HCl added. It is then thoroughly mixed. The bile pigment, which acts as an acid, is set free from its alkali combination by the HCl, and, being more soluble in this condition in chloroform than water, is extracted by the chloroform, the free pigment being insoluble in water. The chloroform is then poured off into another test-tube and equal amounts of water added. One drop of NaOH is then added, transforming the free pigment to an alkali salt, which is again soluble in water. The H_2O solution is then tested with nitric acid, or the chloroform extract may be evaporated in a watch-glass and the crystals studied.

By another method the urine is rendered alkaline with NaOH, or soda, and precipitated as long as a colored precipitate falls with $BaCl_2$ or $CaCl_2$, or the hydroxides of these metals. The yellow precipitate is then filtered off and boiled with alcohol plus a few drops of dilute H_2SO_4. A beautiful clear green solution is obtained. If no pigment is present it is colorless; if chrysophanic acid is present, orange-yellow. This test is positive when other tests are negative.

The bilirubin may also be extracted with chloroform from acid urine (add a few drops of HCl). An emulsion should be avoided, however, by not shaking too vigorously. In case it be necessary to test the urate sediment, and this may contain

all of the bile in the urine, the sediment is dissolved with soda and the solution tested for bilirubin.

Hammarsten's Test.—An acid mixture is used, consisting of 1 part 25 per cent. HNO_3 and 19 parts of 25 per cent. HCl. This reagent is allowed to stand until yellow. To 1 part of this are added 4 of alcohol; this mixture is made fresh before each test. To a few cubic centimetres of this fluid are then added a few drops of a bilirubin solution. At once is obtained a permanent beautiful green color. If more acid be added we get at will the other colors, even the yellow choletelin. But for urine this test must be modified somewhat. Ten cc. of urine are placed in a 15 cc. tube of a centrifuge, a few cubic centimetres of $BaCl_2$ solution added and the mixture centrifugalized for from one-half to one minute. The supernatant fluid is then poured off. 1.2 cc. of the above acid reagent are then added to the sediment, this shaken well and centrifugalized for half a minute. (If $CaCl_2$ be used, this last centrifugalization is not necessary.) A green solution is obtained. The test is very delicate, being positive if there is 1 part of the pigment in 500,000 to 1,000,000 parts of urine.

Huppert's Test.—Ten cc. of urine are made alkaline with soda and $CaCl_2$ added as long as a precipitate is formed. This is filtered in a small filter and washed with water. The filter with the precipitate is then placed in a porcelain dish and acid alcohol (5 cc. HCl in 100 cc. of alcohol) added. This is heated and gives a green to a blue colored solution. This test is advised should indican be abundant or the urine dark in color.

Nakayama's Modification of Huppert's Test.—The reagent used consists of 95 per cent. alcohol, 99 parts; fuming HCl, 1 part; and 4 gms. Fe_2Cl_6 per litre of the above mixture. To 5 cc. of acid urine are added an equal amount of 10 per cent. $BaCl_2$ solution and centrifugalized. The supernatant clear fluid is poured off, to the precipitate are added 2 cc. of the above reagent, and the fluid is then heated to a boil. A green solution is obtained, or a bluish green, which on the addition of yellow HNO_3 becomes violet or red. The test is said to be positive for 1 part of bilirubin in 1,200,000 parts of urine; that is, it is almost twice as delicate as Huppert's test.

The following very important test or modifications of it has gone under four different names, Trousseau's, perhaps, having priority. The urine, acidified if necessary with acetic acid, is mixed with a tincture of iodine, or a contact test made, the iodine tincture being superimposed upon the urine. A fine emerald-green color is obtained (which is not biliverdin but a substitution product of bilirubin with iodine). This test is more sensitive than Gmelin's; it is even more delicate if the tincture of iodine be diluted 1 : 10 with alcohol (hence a 1 per cent. iodine solution) and the urine be overlaid with this. A green ring appearing at once or in one minute will indicate bile (Rosin). In this test there is no confusion with indoxyl. It is said, however, that some normal urines will give a positive test. (Cl or Br may also be used.)

Stokvis's Cholecyanin Test.—This test is a good control if bile be present with much other pigment, but it is not as delicate as some of the above. To 20 to

30 cc. of urine are added 5 to 10 cc. of ZnAc solution. A little soda is added to reduce the acidity. Or, 20 per cent. $ZnCl_2$ solution may be used. It is then filtered. The precipitate contains all of the bile. This is dissolved in NH_4OH. The bile pigment is now in the form of cholecyanin. The solution is neutralized, is of a blue-green color, with a red fluorescence and a characteristic three-band spectrum.

Many of these tests are good. Some when only bile is present, others when many other pigments also.

Certain substances may be in the urine which it is important should not be mistaken for bile, as after the use of rhubarb, senna, and santonin. The change of color of these urines to red on the addition of an alkali should prevent confusion. The color is restored if the urine be again acidified.

Microscopically, it is important to recognize the crystals of bilirubin. They are commonly present in jaundiced urine if concentrated for leucin and tyrosin. The urine is rendered acid with HCl and allowed to stand in the cold. Bilirubin will precipitate out in intensely brown sheaths or rhombs often with rounded edges; their color should prevent any confusion.

It is sometimes desirable TO REMOVE BILE from the urine. This may be done by extracting a urine acidified with HCl with chloroform, or by briefly boiling with a little animal charcoal. This latter method should be carefully used, since other substances, perhaps the one sought for, may also be removed.

$KMnO_4$ in acid solution destroys the bile pigments perfectly. Two drops of HNO_3 or HCl per 1 cc. of urine are added, and 2 drops of 4 per cent. $KMnO_4$. The urine is then warmed and shaken a little.

Bouma [48] recommends the following QUANTITATIVE DETERMINATION FOR BILE: To 10 cc. of fresh urine are added 2 cc. of 20 per cent. $CaCl_2$ solution, and the urine is then almost neutralized with NH_4OH. The slightly acid urine is then centrifugalized, then again, to wash the sediment, then the fluid entirely decanted, and 5 cc. of a mixture of 4 cc. of absolute alcohol and 1 cc. of Obermayer's reagent (1.5 gms. of Fe_2Cl_6 in 1 litre of HCl, sp. gr. 1.15) are added, all poured into a test-tube, and compared with a set of six standard tubes to match the biliverdin which has been formed. If much bilirubin (more than 100 mg.) be present, the urine is diluted with normal urine (thus not diluting the phosphates).

Melanin-Melanogen.—In the case of melanotic tumors this substance or substances (Mörner) may be present in the urine. The chromogen is colorless, but the urine on standing, or after the addition of an alkali or oxidizing agent, turns black, beginning at the top; it may be intensified by adding HNO_3 or Fe_2Cl_6. It is insoluble in chloroform, which prevents its confusion with indoxyl. It may be

[48] Deutsche med. Wochenschr., 1904, No. 24.

present as an amorphous sediment. It is decolorized by boiling with NHO_3.

Rosenbach's Reaction.—The urine is boiled, adding from time to time, drop by drop, strong nitric acid. The urine takes a Burgundy-red color and the foam a bluish-red. The foam must be of this color, since the red of the urine may be due to urobilin. (An excess of HNO_3 gives a yellowish-red to yellow color with a yellow foam.) If, then, soda or ammonia be added drop by drop we get a bluish-red precipitate soluble in excess and a brownish-red solution. This test is said to be due to indigo-red (Rosin), perhaps also skatoxyl-red. It has the same significance as the indoxyl reaction.

Bile Acids.—Glycocholic and taurocholic acids are denied to be constituents of normal urine, as was formerly believed, but they may occur in large amounts in jaundice, especially the obstructive, although none may be present even here, and in toxic jaundice they appear but in traces. Formerly their presence was supposed to speak against the latter, but they are of little value in this differential diagnosis. Their direct detection in the urine is impossible unless 0.5 per cent. of the acid be present.

SEPARATION.—*Thierfelder* recommends that the urine be concentrated to a small volume, the residue extracted with strong alcohol, and filtered. The alcohol is evaporated and the urine precipitated with basic PbAc and NH_4OH. The precipitate is washed with water, dried, treated with boiling alcohol several times, and filtered hot. The filtrate, plus a few drops of soda solution to decompose the Pb salts, is evaporated to dryness, the residue extracted with absolute alcohol, filtered, and a great excess of ether added and allowed to stand. An amorphous precipitate of the sodium salts of these acids is obtained, later crystalline. The precipitate is dissolved in water and tested by Pettinkofer's test.

Tyson Method.—From 180 to 240 cc. of urine are evaporated to dryness on the water-bath. An excess of absolute alcohol is added to the residue and filtered. To the filtrate are added from 12 to 14 volumes of ether, which precipitates the bile acids. They are then filtered off, dissolved in distilled water, and decolorized with animal charcoal.

Pettinkofer's Reaction.—To the solution in a test-tube is added a little cane-sugar and then slowly drop by drop concentrated H_2SO_4, shaking well all the time and warming to 70° C., not over, and cooling if necessary. We get first a precipitate of cholic acid, which redissolves. Then when more H_2SO_4 is added there is obtained, first a cherry-red, then a beautiful purple color, which in eight days is a bluish-red. This color is due to the reaction of cholic acid and the furfurol, which is formed by the action of sulphuric acid on cane-

sugar. The purple-red solution may be diluted with alcohol, and shows a characteristic absorption spectrum. Confusing substances which may be present are albuminous bodies, many bodies easily decomposed by H_2SO_4, many pigments, amyl alcohol, and oleic acid; but in all these the absorption spectrum fails.

Udránszky's Test.—This is the best test. Furfurol is used directly. To 1 cc. of the solution to be tested is added 1 drop of a 0.1 per cent. watery furfurol solution. This is underlaid with 1 cc. of concentrated H_2SO_4 and cooled to restrain the reaction. In the presence of only 0.033 mg. of cholic acid is obtained a red color which after standing becomes a blood-red. If 0.05 mg. be present, one gets a distinct absorption line in the spectrum. The spectrum must always be examined for confirmation. A 10 per cent. cane-sugar solution will give as good a test as a 0.1 per cent. furfurol solution. The red color must have a clearly bluish tinge.

If the cane-sugar be used in excess it is burned to a brown or a black. An excess of furfurol gives an orange color. Oxidizing bodies prevent the reaction.

Strassburger's test may be sometimes applied directly to the urine. It is useless, however, since normal urine will give a confusing color, and jaundiced urine is not suitable because of its color. This test is easy if a little bile be added to normal urine, but it is almost impossible to apply in a jaundiced urine. To the urine was added a little cane-sugar and then filtered; the filter paper was dried, and one drop of pure sulphuric acid added. In about a quarter of a minute is seen a violet spot. This will detect 0.3 gm. in 1 litre.

Skatoxyl and indoxyl will give a violet color, and with concentrated normal urine beautiful positive tests may be obtained.

If one wishes to be sure of bile acids they must be isolated as lead salts, the other tests being unsatisfactory.

Hay's test for bile acids is said to be very easy, sensitive, and accurate, being given by no other body occurring in the urine, and more delicate than the Pettinkofer test. On the surface of the urine (which has been cooled, if necessary, to, at the highest, 17° C.) is sprinkled a little finely powdered sulphur. If the sulphur sinks at once, it indicates 1 : 10,000. If it sinks after shaking gently and waiting one minute, 1 : 40,000. It is given by even 1 : 120,000. The bile salts are said to lower the surface tension.[49]

Diazo Test.—Certain diazo bodies combined with aromatic compounds give a colored reaction. A test depending on this is recommended by Ehrlich for clinical use. What body or bodies give it in the urine are unknown, but the empirical value of the test is granted. Since there are a great many diazo tests for various bodies, one must be careful in modifying this one of Ehrlich.

Fluids:

(1) One-half per cent. $NaNO_2$. This should be quite fresh.

[49] Beddard and Pembrey, Brit. Med. Jour., March 22, 1902.

(2) Five parts of sulphanilic acid, 50 of HCl, 1000 of distilled water.

To 250 cc. of the second are added 5 cc. of the first solution. Only a fresh mixture (not over one day old) should be used. Equal parts of the urine and this mixed reagent are shaken together until considerable foam is produced and ammonia is then quickly added in excess; usually it is added drop by drop, although we are warned not to thus modify in the least the original technic. If the test be positive, the urine will take an intense red, the foam a more or less brilliant rose-red color. A brown color is often obtained in normal urines, and unless the color is a definite rose the test should be considered negative; a salmon tint is not positive. If a positive test be allowed to stand, a precipitate should form, on the upper surface of which is a zone of dark greenish-black, or violet. In case the color of the foam is doubtful,—if, for instance, after shaking the red disappears,—we are recommended to wait twenty-four hours for this precipitate. Others consider that the sediment is a less delicate indicator than is the color of the foam, and is not essential to a positive test, hence neglect it.

Some say the red color must remain in the fluid until the foam is gone.

In Green's modification 100 parts of solution 2 are used with 1 part of the nitrite. This renders the test more delicate, since fewer unexpected positive results are obtained. With strong enough reagents every urine will react positively.

Sulphanilic acid is usually used for the reagent, and yet Zunz prefers the paramido-acetophenol of Friedenwald's formula: [50]

Paramido-acetophenol, 50 gms.
Conc. HCl, 50 cc.
Water, q. s. ad 1000 cc.

Four drops of a 0.5 per cent. solution $NaNO_2$ are added to 10 cc. of the above solution, and this to 10 cc. of urine. The mixture is then shaken, about 3 cc. of ammonia added, and the color of the foam observed. It is more delicate and intense than the sulphanilic acid. This author prefers to add the ammonia all at once, and not drop by drop. He considers the foam as the more important, and the precipitate of less value, not sufficient to make the test positive should the foam be negative. The disturbing bodies may many of them be removed by shaking the urine out with amyl alcohol, which must itself be then driven off on the water-bath.

The test is further modified by Guillemin. Fifty cc. of HCl are added to 1 litre of saturated aqueous solution of sulphanilic acid.

[50] New York Med. Jour., 1894, p. 745.

Two and one-half cc. of urine plus an equal amount of this reagent are mixed, and then are added two drops of $NaNO_2$ solution. It is then well shaken and from 7 to 10 drops of NH_4OH added.

Lamanna makes the solutions with absolute alcohol instead of water.

Solution I.	50 cgms. sulphanilic acid;
	5 cc. HCl;
	100 cc. absolute alcohol;
	5 cc. glacial acetic acid.
Solution II.	50 cgms. $NaNO_2$;
	50 cc. absolute alcohol.

To 5 cc. of urine is then added 1 cc. of NH_4OH. The reagent mixed as in the other tests is then added drop by drop. If the test is not positive, a few more drops of the $NaNO_2$ may be added.

Several methods of QUANTITATIVE DETERMINATION have been attempted. König places in a burette 25 cc. of filtered urine plus 5 cc. of NH_4OH. In a second burette is a mixture of 50 cc. of the sulphanilic acid solution and 1 cc. of $NaNO_2$ solution. Into a flask are measured 5 cc. of the urine solution, and then from the other burette the mixture is added drop by drop until a red color appears in the foam and fluid which just persists after shaking.—Nizzoli determines it quantitatively by diluting the urine until the test is just positive. This is the method preferred by Zunz, who considers, however, that the determination takes more time than it is worth.

The urine soon loses its property of giving a positive test, but after a few days of ammoniacal fermentation the test reappears.

If necessary to keep the urine several days before testing it, ether may be added.

Some prefer to concentrate the urine on a water-bath to a syrup (Michaelis) and get a positive test in some cases in which the urine gave none. Zunz has done the most of his careful work with such concentrated urines. That this does not always help matters has been shown by Imhoff, who found in the experimental tuberculosis of rabbits that the concentrated urine may give a brown foam, but if diluted to its previous volume the foam becomes a brilliant red. In the case of the human urine similar observations have been made by Dr. Hirschfelder, who tests the undiluted and the diluted urine as a routine. In work done in this clinic we have been in the habit of testing the diluted, the concentrated, and the unaltered urine. I am told that certain urines giving no test according to usual technic give a good one if only one-half volume of reagent is used.

What the body is which gives the red color when combined with a diazo is not known. One of the interesting recent suggestions is that of Bondziński, who found alloxyproteinic acid in all normal urines

and since it will give the test suggests that it is the cause. Clemens replied that this was not the important body since the body giving it is sulphur-free.

Occurrence.—In health the test is never positive. Ehrlich has divided the diseases into four groups. In the non-febrile diseases it is rarely positive; in advanced heart disease, chronic hepatitis, carcinoma, especially of the pylorus, leukæmia, marasmus senilis, malarial cachexia, tuberculous abscess.

Febrile Diseases.—Of these there are three groups:

(1) Those in which the test is almost never given,—*e.g.*, acute articular rheumatism and meningitis.

(2) Diseases in which it may or may not be positive,—as pneumonia, scarlet fever, diphtheria, erysipelas, and phthisis.

(3) Those in which it is almost constantly present,—typhoid fever and measles.

Lobligeois in scarlet fever found the test positive in 42 of 52 cases, and in but 3 of 137 cases of diphtheria. He considers it therefore important in the diagnosis of cases of diphtheria with a scarlatinal rash —*e.g.*, the serum erythema. Brunschwig found that in children the reaction is always positive in typhoid, often in scarlet fever, quite often in measles, rarely in pneumonia, and never in whooping-cough. Tropea and Brancati consider the test is not very valuable, since it occurs so variably in some diseases, so often in others, and, they claim, in some normal persons. In disease they suppose it to depend upon the virulence of the organism and the products of the breaking down of body tissues.

Ehrlich considers that in the first two groups of fevers, those in which it is almost never and those in which it is sometimes present, the positive reaction means a poorer prognosis. In suspected typhoid fever it is agreed that its continued absence speaks strongly against that diagnosis, also that its reappearance allows of a differentiation between a relapse or recrudescence of the typhoid and a fever due to a complication. Johnson found it present in over 80 per cent. of his cases. Montier found the test present in all cases of the pulmonary type of typhoid fever. Déléarde and Hautefeuille [51] found the test positive in severe cases of typhoid fever, and considered that drugs had no influence, nor did it bear any relation to intestinal putrefaction. Phenol is an important body to inhibit its appearance. Others consider that in typhoid fever it is of no value, since early when most wanted it is so often negative.

In phthisis it is supposed to indicate a bad prognosis, although the previous opinion of Michaelis that such cases were always fatal is not borne out by the experience of others. Boissière found it in 18 of 130

[51] Compt.-rend. Soc. Biol., vol. liv. p. 279.

severe cases. There is some reason to think that in tuberculosis it is due not to the tubercle bacillus, but to some secondary infection.

It cannot be used to distinguish between typhoid fever and miliary tuberculosis, since in the latter it is so often present. It occurs also in puerperal fever and in actinomycosis of the lung.

The work of Zunz [52] is of particular interest to us, since it seems to have been done with exceptional care. His conclusions are that the value of the test is limited to the early diagnosis of typhoid fever and to the prognosis of tuberculous pneumonia, yet in the latter disease its presence may not mean a hopeless one; that it is of diagnostic value in early cases of measles, and speaks in favor of tuberculosis in cases of peritonitis, pleurisy, and nephritis; it is often present in erysipelas; if present, the prognosis in a case of cancer or sarcoma is more serious ; in cases of pneumonia and pyothorax (non-tuberculous) the test means merely disturbed metabolism; in certain cardiac affections it speaks in favor of a reserved prognosis; in conclusion, it is a useful test, but its value has been much exaggerated.

Many consider that the ingestion of certain drugs prevents the test, as, for instance, phenol, salol, benzonaphthol; not that these bodies inhibit the formation of substances giving the test, but that they themselves unite with the reagent, thus preventing the reaction, and if they are extracted with amyl alcohol the test is positive. Zunz does not agree.

Plezl [53] found it present in typhoid from the middle of the first to the end of the third week; in measles, before the eruption and during the onset. His suggestion is that apart from these conditions it occurs in streptococcus septicæmia, which explains its presence in the angina of scarlet fever, advanced lung tuberculosis and other forms of severe tuberculosis, and in conditions with a general septicæmia.

We find the test very valuable. When present it is strong evidence in favor of typhoid fever. In our typhoid cases the test very soon is negative, the result of the diuresis we encourage.

Ehrlich's "Egg-Yellow Reaction."—Ehrlich has called attention to a somewhat characteristic reaction in cases of pneumonia before and during the crisis. If the diazo test be tried, the urine and the foam take a yellow color before the addition of ammonia. After it is added the color changes to a lighter yellow. Ehrlich ascribes the reaction to the urobilogen formed from the urobilin of the exudate, and thinks that it predicts the crisis. Others think its value still uncertain.

Ehrlich's Dimethylamidobenzaldehyde Reaction.—This is another color test proposed with the hope it would turn out of value. Dimethylamidobenzaldehyde is dissolved in equal parts of conc. HCl and H_2O to make a 2 per cent. solution. From 5 to 10 drops of this are added to a few cubic centimetres of urine in a test-tube. This is then agitated a few minutes or set aside, and

[52] Bull. de l'Acad. roy. de méd. de Belgique, ser. iv., t. xiv. p. 553.
[53] Wien. klin. Wochenschr., 1903, No. 31.

then the color noted. Normal urines give a greenish-yellow, but some pathological ones a distinct cherry-red color, which constitutes a positive test. This occurs in a variety of conditions, including phthisis, typhoid, and chronic enteritis. Fresh urine must be used and not heated. Nothing of value has resulted as yet.[54]

FERMENTS

Ferments.—Several ferments have been demonstrated in the urine in health and in disease, in amounts depending on the general condition of the patient. The most important of these is PEPSIN. To demonstrate, pure fibrin is allowed to stand for several hours in the fresh urine. This will absorb a great deal of the pepsin. The fibrin is then removed, placed in dilute hydrochloric acid and this in a thermostat. If digestion occurs in acid medium pepsin is demonstrated. Matthes[55] has shown that a certain amount of the pepsin is reabsorbed. TRYPSIN, it is said, has been found, but this has not been confirmed. A DIASTATIC FERMENT surely occurs in some cases and rennin as well. It is claimed that there is a ferment which breaks up the urea molecule forming ammonia bodies. Such ferment, however, needs further demonstration.

Clinically, pepsin has been found absent in typhoid fever, and diminished gastric secretion as in cancer.[56] The question whether such ferments occurred in the blood or were formed by the secreting cells of the mucosa, Dell' Scola[57] decided in favor of the former. He reports them diminished, even absent, in severe disease of the nervous system, especially those with convulsions and loss of consciousness.

Lipase[58] is not present normally or only in traces. It is present in jaundice, perhaps in traces in diabetes mellitus, but is found especially in those conditions with fat necroses (in dogs after mechanical injury of the pancreas, after tying the pancreatic duct).

METHOD (KASTLE-LOEWENHART).—In each of three flasks are measured 5 cc. of urine. The second flask is boiled. To the third are added 3 drops of phenolphthalein (1 per cent.) and it is titrated with tenth-normal NaOH till faintly pink. This amount of alkali is then added to flasks 1 and 2. To each of these is then added 0.25 cc. of ethylbutyrate and 0.1 cc. toluene, and they placed in a thermostat at 39° C. for twenty hours. An amount of tenth-normal HCl, which is 0.5 cc. more than the amount of tenth-normal NaOH previously added, is then added to each, they are shaken out with 50 cc. of ether and 25 cc. of alcohol, 3 drops of the phenolphthalein solution are

[54] Simon, Am. Jour. Med. Sci., 1903.
[55] Arch. f. Exp. Path., 1903, vol. xlix. p. 107.
[56] See Friedberger, Giessen, 1899.
[57] Centralbl. f. inn. Med., 1902, No. 14.
[58] See Hewlett, Jour. Med. Research, 1904, vol. vi. p. 377; also Garnier, Compt.-rend., 1903, vol. v. p. 1064.

added to the ether extract and the amount of butyric acid split off titrated with tenth-normal KOH.

In case 5 cc. of urine for each flask is not available the figure obtained from the smaller amount is calculated for 5 cc., using the formula that the amount of ferment action varies as the square root of the amount of ferment present.

CARBOHYDRATES AND ALLIED BODIES IN THE URINE

A small amount of carbohydrates is a normal ingredient of the urine. Three have been demonstrated,—glucose, animal gum, and isomaltose. Related bodies are also present,—the paired glycuronic acid compounds, chondroidin-sulphuric acid, nucleinic acid, and the mucoid of the nubecula, sometimes also pentose. The total output of these carbohydrates measured as glucose amounts to from 2 to 2.23 gms. in twenty-four hours. Of glucose there is normally from 0.38 to 0.62 gm. in twenty-four hours (Naunyn, 0.4 to 1.4 gms.).

The TOTAL CARBOHYDRATES, fermentable and unfermentable, may be determined as the benzoylester. The urine is made alkaline with NaOH and the phosphates filtered off. To the filtrate in a flask are added 4 cc. benzoylchloride per 100 cc. of urine, and 40 cc. of 10 per cent. NaOH, and shaken gently for ten minutes (to avoid emulsion), then vigorously for twenty to twenty-five minutes, till all odor of the benzoylchloride has disappeared. It is allowed to stand a few hours, not over night since the precipitate gets sticky and will not filter well, then filtered, the precipitate washed, dried over H_2SO_4, and weighed.

The *assimilation limit* is an interesting as well as important conception in functional diagnosis. By this is meant the minimum amount of sugar the ingestion of which by mouth is followed by the excretion of a slight amount in the urine. A lesser amount the body can either oxidize or warehouse. Hofmeister found that galactose and lactose passed the most readily into the urine; dextrose, lævulose, and cane-sugar much less so.

After a meal of about 200 gms. of glucose a normal person excretes as a rule none, or seldom more than 1 gm.; some persons none after 300 gms. Some normal persons will excrete a little after smaller amounts; for instance, of 50 gms. It occurs with greatest ease if the sugar is given on an empty stomach. Hunger lowers the assimilation limit considerably; pregnancy also does the same. Diseases lowering the limit are cirrhosis of the liver, cerebral disease, poor nutrition, fatty liver, phosphorus poisoning and infectious diseases, certain neuroses, exophthalmic goitre, and any condition causing diuresis.

Naunyn has divided cases of alimentary glycosuria into those following the ingestion of starch, and those which follow the ingestion of sugar. The former denotes a disturbed metabolism very nearly if not always diabetic, for no matter how much starch (even 600 gms.) is

ingested, no glycosuria should follow. This perhaps may be explained by the slow transformation, hence absorption, of the products of starch digestion.

All grades of weakness of sugar metabolism occur, from those with a slight lowering of the assimilation limit, to those in which glycosuria follows large doses of starch, and, finally, cases of true diabetes mellitus.

The assimilation limit may be conveniently tested by the following test meal (Naunyn): At breakfast, coffee and milk (about 250 cc.), and 80 to 100 gms. of bread are eaten. In about two hours 100 gms. of dextrose are taken at one time. Thus the sugar is not taken on an empty stomach. If a measurable glycosuria results the limit is pathologically lowered. If 1 per cent. of sugar is present the suspicion of diabetes is very pressing. The sugar excretion begins in about one hour, reaching maximum in from two to four hours, and lasts at the longest but eight to ten hours. If glycosuria follows a meal rich in starch foods, not glucose, the suspicion of diabetes is very strong indeed.

In certain cases with an apparently lowered assimilation limit the suspicion of diabetes may be unjust, since it has been shown that if the sugar reaches the lower part of the small intestine it seems to be absorbed by the lymphatics, hence does not pass through the liver but at once into the circulation and hence is excreted.

The *hunger diabetes* of Hofmeister is very interesting. He found that if dogs under close confinement be kept on a poor diet, not starved, a certain number of them soon become diabetic, excreting 30 per cent. of the starch of their food as sugar. We presume that almost every one who has had much experience in metabolism experiments with dogs has found illustrations of this form of diabetes. Naunyn made the prophecy that this would soon be found to explain the glycosuria of certain chronic diseases of man, the disease bringing about a condition of malnutrition. Soon after this the report of Hoppe-Seyler [59] of ten cases of temporary glycosuria in tramps who had been under very unsuitable hygienic and dietary conditions, and which disappeared in twenty-four hours, after their physical condition had improved somewhat, was the first confirmation of this prophecy.

Glycosuria.—That normally a small trace of glucose is present in the urine can be proved by isolating the glucosozone from large amounts of urine. Quantitative reduction tests before and after fermentation of the urine also indicate this body.

Theoretically, glucose appears in the urine: (1) When in the blood it has reached 0.3 per cent. or over, that is, a distinct hyperglycæmia. A hyperglycæmia may be due to the ingestion of more

[59] Münch. med. Wochenschr., April, 1900.

sugar than can be warehoused or to the accumulation in the blood of glucose which the body cannot use, the function of the kidney being to excrete any excess over the physiological percentage of the blood. (2) When there is on the part of the kidneys a diminished ability to retain it, *e.g.*, after phlorizin injection. (3) Some compound of glucose rendering it unfit for use.

Clinically, the cases may be grouped according to Hammersten as follows:

(1) Those with a lowered assimilation limit. In this group Seegen put his cases of mild diabetes. In such there is no glycosuria on a carbohydrate-free diet, the liver being able to warehouse well the sugar formed in the body.

(2) Those with an excessive amount of glucose formed in the body at the expense of glycogen and other bodies. This occurs after certain experimental lesions of the brain and perhaps after certain cerebrospinal diseases. Perhaps in this group also are to be included CO, curare, strychnine, and morphine poisoning. The source of the sugar is probably the albumin, since sugar appears in the urine only if the animal has had enough albumin. During albumin-hunger, even on a rich carbohydrate diet, no sugar is found.

(3) Cases in which the body cannot use the glucose, and it therefore collects in the blood. Severe cases of diabetes mellitus belong here. Such are not due to the inability of the body to burn the sugar, since the combustion ability of the patient has been proved normal. Levulose is well used for a while. Usually diabetics are unable to use the dextrose molecule alone, perhaps to produce the preliminary splitting of this molecule. All urinary changes are merely the result of this. According to Opie these cases belong to the following group.

(4) After disease or removal of the pancreas. In dogs the glycosuria may reach 10 or 22 per cent.; the animal lives not over four to five weeks. In such experimental cases practically all the sugar ingested is excreted, and in a quite constant ratio to N (2.8:1); in analogous cases in man not quite all is excreted. In some cases is found atrophy of the pancreas or degeneration limited to the islands of Langerhans.

(5) Glycosuria follows oxygen starvation due to any cause; suffocation, the death agony; certain poisons, as CO, curare, and amyl nitrite; narcotics, as ether, chloroform.[60]

(6) Certain poisons, including morphia, strychnine, and cocaine;[61] fusel oil, $HgCl_2$, acids. In this connection the work of Herter[62]

[60] See also Brown, Johns Hopkins Hosp. Bull., May, 1900.
[61] See also Neubauer and Vogel, p. 92.
[62] Am. Med., 1902, p. 771.

is interesting, showing that the local application of reducing substances to the pancreas (adrenal extract and various poisons, H_2S, KCN, H_2SO_4) causes glycosuria. Blum had shown that a substance from the adrenal caused glycosuria, and Metzger that this depended on hyperglycæmia. Herter thinks that it is the oxygen deprivation which is at fault.

(7) After severe cooling of the body.

(8) Renal diabetes. After caffeine or theobromine, or any diuretic which increases the secretion of the kidney. Phloridzin diabetes shows the possibility of renal diabetes, and these are the only cases without hyperglycæmia. In some cases of chronic nephritis one sees diabetes, but, as a rule, the former is secondary to the latter, and as the nephritis develops the glycosuria diminishes, the so-called "cure by Bright's disease."

After the transfusion of normal salt solution; after the injection of sugar into the blood; after insults and injuries to the liver, well seen in animal experiments; a similar connection may explain the rare cases of cirrhosis of the liver with glycosuria; after diseases and injuries of the central nervous system, the best illustration of which is in animals, the piqûre of Claude Bernard, causing hyperglycæmia of even 0.7 per cent. due to the inability to retain the glycogen, which lasts from six to forty-eight hours, and a glycosuria which may reach in rabbits even 6 per cent.

In man a similar glycosuria follows apoplexy, transient, as a rule, beginning in two hours and lasting even six days, reaching 1 to 2 per cent.; brain tumors, especially of the base; dementia paralytica commonly; epidemic cerebrospinal meningitis; tabes; multiple scleroses; diseases of the sympathetic nervous system; severe trauma of the skull, in which case it is usually permanent, beginning at once or in a year, and mild as a rule, some with an interesting relation to diabetes insipidus, beginning as this and ending as mellitus; functional neuroses; psychical causes; exophthalmic goitre; gout; arteriosclerosis; obesity.

In pure diabetes no gross lesion is found (but see page 159). Such occurs especially in the young and includes nearly all severe cases.

QUALITATIVE TESTS FOR GLUCOSE

Trommer's Test.—To a test-tube half full of urine is added about one-third volume of 10 per cent. NaOH or KOH and then a 10 per cent. solution of $CuSO_4$ in drops, until a few flakes of $Cu(OH)_2$ do not disappear on slightly shaking. The upper layer of the urine is then warmed, when at once a precipitate yellow or red in color appears at the top. When this appears the heating should at once be stopped. The reduction and the precipitate will spread through the fluid from

above downward. The urine should always be examined fresh, and much albumin removed in all cases.

The reaction is as follows: If to pure water be added KOH and then the $CuSO_4$, the first drop of the latter will cause a precipitate of $Cu(OH)_2$ [$CuSO_4 + 2NaOH = Na_2SO_4 + Cu(OH)_2$]. These flakes of $Cu(OH)_2$, on heating, will blacken, since $Cu(OH)_2$ $2CuO$ is formed. If glycerin or the tartrates be added to the water, all of the $Cu(OH)_2$ is dissolved to a blue solution, which will not blacken on heating as it does if undissolved. If, instead of these, glucose be added to the water, the same blue solution of the $Cu(OH)_2$ is obtained. This, however, on warming is reduced, and a yellow or red precipitate falls. In the case of glucose the body giving the bright blue solution is $C_6H_{12}O_65Cu(OH)_2$.

In the normal urine certain bodies are present which, like glycerin et al., will dissolve the $Cu(OH)_2$. Such are the ammonia bodies, both those preformed and those resulting from boiling an alkaline urine, and albumin if present. These, however, are present not in sufficient quantity to give a clear blue solution, and only 3 to 5 drops of the $CuSO_4$ can be added before some remains as a precipitate and that dissolved gives only a slight greenish color to the solution. If, however, to the normal urine or in the reagents, glycerin, the tartrates, or more ammonia be added, more or all of the $Cu(OH)_2$ will be dissolved, giving an azure blue solution varying in depth with the amount of $Cu(OH)_2$ added.

But the normal urine also contains reducing bodies which will reduce the copper on warming. Such are uric acid, the glycuronic acid compounds, pyrocatechin, and bile pigments if present, and always a trace of glucose. But the sum of all these equals about 0.5 per cent. if expressed in glucose. These bodies, when present in normal amount, will reduce some of the copper and give a yellowish solution, a dirty not a clear yellow, and a little will be carried down with the phosphate precipitate, tingeing it. If these bodies normally present be increased, a definite precipitate, hence a positive test, may result. But uric acid does not reduce at a temperature of from 60° to 70° C., and creatinin reduces much only after long boiling, although there is a little reduction at 60° C.; hence, as no high temperature is allowable in this test, these bodies should not confuse. They are very important, however, since they hold in solution the small amount of the suboxides which is always formed. The ability to hold in solution these reduced suboxides in the normal urine is much greater than its reducing ability, and hence glucose may be added to normal urine up to almost 0.5 per cent. before any precipitation occurs. The bodies holding the suboxides in solution are uric acid, creatinin, the ammonia salts, and albumin if present.

In glycosuria we have a great increase in glucose, the chief reducing body, and because of the polyuria a relative decrease in the amount of those bodies preventing the precipitation of the cuprous salts. In performing the test it is particularly important that the excess of copper should not be added since the black oxide will cover the precipitate of the cuprous salts. Normally 3 to 5 drops of the $CuSO_4$ are sufficient to give a blue precipitate. In case sugar is present, however, the addition must continue until the first flakes of $Cu(OH)_2$ remain. The test is positive only when a yellow or red precipitate falls, yellow $Cu_2(OH)_2$ in a relatively weak alkaline, red Cu_2O in a strongly alkaline solution. (Neumayer[63] says it is the creatinin of the urine which causes the amorphous yellow rather than the crystalline red precipitate such as pure glucose solutions give.) When much alkali is used the creatinin is transformed to creatin. If much sugar is present, metallic copper may be deposited on the glass (it is often a problem to clean such test-tubes, and strong nitric acid is recommended). In case under 0.2 per cent. sugar is present there will be no precipitate, and yet even then the test may be very suggestive, since the yellow solution will be of such a clear brilliant color. Again, the precipitation should occur under the boiling point or when the urine is just brought to that point to exclude the reduction by those bodies normally present.

For a successful test the proportions of the reagents should be rather accurate.

[63] Deutsch. Arch. f. klin. Med., 1900, vol. xlvii. p. 197.

Since one part of sugar can reduce about five parts of $Cu(OH)_2$, as nearly this amount of copper as possible should be in the solution. Glucose alone, however, cannot dissolve nearly as much as it can reduce, and hence in the copper tests other than Trommer's glycerin, ammonia, or the tartrates are addded to the reagents that this amount may be at the disposal of the glucose. The optimum relation is 1 part of glucose to 5 (3 to 7) of $Cu(OH)_2$ and 11 of NaOH. The excess of this last reagent is necessary, since the temperature of reduction depends directly upon it. If very little be present, a reduction may require hours of boiling. If but two parts of NaOH are present to one molecule of sugar, a few minutes' boiling is enough, while with an excess it is not even necessary to raise it to the boiling point to get a fair reduction.

Again, the best chance of a precipitation occurs when there is present a minimal amount of those bodies which hold the reduced copper salt in solution. For this reason it is advised by many, as a matter of routine, to always dilute the urine about 1 : 5, this strong dilution ruling out the influence of these other bodies in a much greater proportion than it diminishes the reducing power of the glucose.

If to a strong solution of glucose be added strong NaOH or KOH, then a little copper and the whole heated, a yellow or yellowish-brown or a dark-brown solution is obtained, the color varying with the amount of sugar and alkali, since there is not enough copper in solution and some sugar is destroyed as in the Moore test. This color plus that of the suboxides gives a color which much surprises the students. It is avoided by trying the test anew and adding a great deal more copper.

The best of the results are obtained if the copper be added before the alkali. This is a method we have noticed Naunyn to use. More urine, however, must be added in case it is found that too much copper was used.

In the Trommer's test the fluid must decolorize as the precipitate forms and before the boiling point is reached. The precipitation must occur while it is still hot, and not after it cools down, as often occurs. When, however, but a trace of sugar is present, the precipitate may come only after long boiling or after cooling. This test is not positive. The brilliant color of the yellow solution may indicate sugar, but in such a case, if the urine be much diluted, the precipitate may occur in the desired manner. In order to rule out a mistake arising from long boiling, some add to the boiling urine one-third volume of cold NaOH and then copper. Some of the sugar, however, will have been destroyed by the alkali before the copper is added, and hence the test is not nearly as delicate. Since a normal urine reduces some copper, and could more if it were in solution, ammoniacal urines may give a good precipitate. It is also true that the great excess of NaOH will dissolve some of the $Cu_2(OH)_2$, and in case of strong ammoniacal urine all of the cuprous salt may be held in solution. It does no good to add more copper, since the sugar has by this time all been destroyed. In a normal urine it is possible sometimes to get a positive test by adding an excess of NaOH and too much copper.

After warming there may be a clear yellow solution in a normal urine or a grayish-green shimmer due to a slight precipitate of the copper compounds of the xanthin bases and uric acid. The copper precipitated by sugar is crystalline, that by the xanthin bases is amorphous.

In all copper tests albumin does not hinder reduction, but does the precipitation, and hence must be removed unless but a trace is present, which may then be disregarded.

The phosphate precipitate stained slightly yellow by the $Cu_2(OH)_2$ formed even in normal urine often deceives.

The urine may give a reduction when the glycuronic acid compounds are increased. Such follows the use of chloral hydrate, chloroform, morphine, camphor, phenol, resorcin, thymol, and menthol. A positive reduction is obtained sometimes after the use of salicylic acid, benzoic acid, chrysophanic acid, oxalic acid, salol, thallin, santonin, copaiba, rhubarb, sulphonal, chloroform, acetphenetidin, glycerin;

after poisoning with KOH, H_2SO_4, and arsenic. In alkaptonuria the test is positive. Saccharin hinders the reduction. In addition to the reducing substances of non-diabetic urine mentioned are to be added allantoin, mucin, pyrocatechin, hydrochinon, urobilin, perhaps also indican.

We insist that the Trommer's test, although it is used but very little in practice in this country, shall be the one upon which the students shall practise the copper tests. The reason for this is that all steps in the process are evident, and the chances of error are very apparent, hence the difficulties of copper testing can be well learned through it. It is not so delicate as the Fehling's, and yet we have been interested to see those who have had the greatest experience in sugar work use this qualitative test as a routine matter. The reason for this is that it tells more than does the Fehling's, indicating the presence or the absence of certain bodies and in a rough way the amount of sugar that is present. If, for instance, the undiluted urine gives a barely positive test, 0.2 per cent. of sugar may be assumed, and from the amount of copper necessary to add for a good precipitation a rough approximation may be made.

Fehling's Test Solution.—In Fehling's (see page 170) Rochelle salt is used that there may be a maximum amount of copper in solution, at least 5 of $Cu(OH)_2$ to 1 of glucose, and hence the optimum chance of precipitation without the possibility of a black precipitate. Fehling's solution is made from two fluids which must be kept separate, each quite permanent.

These must be mixed in equal quantities for each test, or at least the mixed solution should not be kept over one day, since an old one may reduce on boiling. Equal amounts of these two fluids are mixed and brought to a boil. The urine is then added in small amounts until a precipitate is obtained, the amount of urine, however, never exceeding that of one of the solutions. The precipitate should appear at once. The mixture may be brought again to the boil, but prolonged boiling should be avoided; also a precipitate which forms after the urine has been allowed to stand does not necessarily indicate sugar. As usually performed, the amount of urine is added to the boiling Fehling's in one amount, yet by slowly adding one can guess pretty accurately the amount of sugar present. The test shows 0.08 per cent. of glucose. Although more delicate, it should be remembered that this test has all the faults of the Trommer's. In normal urine there is always more or less reduction. Error from this is much removed by always diluting to specific gravity 1005 (Zeehuisen), and in general the test is best performed by using a much diluted urine.

Almén-Nylander's Test.—The solution consists of Rochelle salt 4 gms., dissolved in 100 cc. of 10 per cent. NaOH (sp. gr. 1015) warm, and saturated with bismuth subnitrate (about 2 gms. are necessary). When cooled it is filtered and kept in a dark bottle. The solution is permanent for years.

To the urine is added one-tenth volume of this reagent. The mixture is then boiled from two to five minutes. If sugar be present, the fluid will turn black and a black precipitate of metallic bismuth will settle. Should it become black after cooling, the test is not necessarily positive. If only a trace of glucose is present, the white sediment of phosphates may be only slightly gray, especially on its upper surface. The boiling should be continued for five minutes, not less, since only too often will the urine suddenly darken contrary to the expectations of the observer. Since it is difficult to boil this urine so long (by the watch), it is much better to leave the tube in a boiling water-bath. If only a trace of sugar be present, the amount of reagent used, one-tenth volume of the urine, must be accurately measured. If this test is negative we may be sure no sugar is present. If faintly positive, the test must be confirmed, since bismuth is also reduced by certain paired glycuronic acid compounds sometimes present. This test is very delicate; in fact, is given, some say, by normal urine (14 per cent. of cases). Uroërythrin may deceive, since it simulates the test; also hæmatoporphyrin. Concentrated urines may give a positive test. The test will indicate 0.05 per cent. (others say 0.025 per cent.). Any increase or diminution in the alkalinity of the fluid injures the delicacy of the test, hence it should be applied carefully in an ammoniacal urine. If the sugar is over 0.2 per cent. the yellow color of the Moore test is first seen. Rhubarb and senna will give a reduction, but before heating it will be noted that the fluid takes a brownish-red color. The test is positive after salol, benzol, sulphonal, trional, antipyrin, kairin, much quinine, eucalyptus tincture and oil of turpentine. It is also positive after a person has eaten asparagus, a fruitful source of error. All of the albumin should be removed unless it be but a mere trace, since the Bi_2S_3, if precipitated in considerable amount, is of a brownish color; if very little, a red. Ammoniacal urines are disturbing, since the NaOH replaces the ammonia, which is volatilized, leaving the solution not alkaline enough. This test is very valuable, since it confirms Trommer's splendidly, it not being given by uric acid, creatinin, pyrocatechin, hydrochinon, nor the alkapton bodies; in fact, by the most important disturbing substances.

Fermentation.—This is necessary to prove that the reducing body is a sugar of three or a multiple of three carbon atoms (yet not all of these ferment). Fresh active yeast should be used. A piece about the size of a pea is added to the urine, which is then gently shaken (if shaken too hard the amount of air in solution will be increased and afterwards give a bubble suspiciously large), and the urine then filled into a fermentation tube. This is let stand at the optimum temperature of from 15° to 34° C., and the presence of gas determined in a few hours. Two control tests should always be made: the one with normal

urine to which a little glucose is added, to prove the activity of the yeast; another of normal urine alone, to prove by the absence of gas that there is no self-fermentation of the yeast. Above 45° C. there is no fermentation. The rapidity depends to a certain extent on the amount of the yeast. The amount of gas formed from a given amount of sugar, however, depends on the age of yeast, there being less formed by an older one. The maximum production of CO_2 (46.5 per cent. of the sugar) is obtained only when to one part of sugar is added not more than one-half of fresh yeast. If more yeast be used self-fermentation may result. This test indicates from 0.1 to 0.05 per cent. when boiled urine is used.

Some consider it necessary to prove that the gas which collects is CO_2, by dissolving it in NaOH, and that alcohol is formed by distilling the fluid and forming iodoform. To exclude bacteria the result must be rapid, that is, within a few hours. Or it is better to add NaF to 1 per cent. Many recommend to boil the urine first about ten minutes to sterilize it and also to free it from air, and to use a yeast treated with NaF. If the result be doubtful, the test may be repeated, trying a new yeast. Tartaric acid may also be used to inhibit bacteria. This is especially good in case the urine be weakly acid or alkaline.

If only a trace of glucose be present, there may be no CO_2 seen, since the urine can dissolve some. In this case acidify with tartaric acid and ferment for twenty-four to forty-eight hours, then again try the bismuth test. If now negative, glucose was present.

Phenylhydrazin.—This test is the court of last appeal in the recognition of those carbohydrates which form with phenylhydrazin osazones of definite crystalline shape and with a definite melting point. In any case albumin must be removed, for it hinders crystallization. Ten cubic centimetres of urine are precipitated with a few drops of concentrated PbAc and filtered. One drop of acetic acid is added (or enough to acidify), then a piece of HCl-phenylhydrazin the size of a pea, and of NaAc the size of a bean. The tube is then boiled in a water-bath from one to two hours, its contents then filtered hot, the tube returned to the water-bath, which is allowed to cool down slowly. If much glucose be present there will be a deposit of yellow crystals, needles arranged in sheaths. That the test may succeed, the sugar should be to the phenylhydrazin and the NaAc as 1 : 2 : 3.

V. Jaksch recommends the following: To a test-tube containing 6 to 8 cc. of urine are added two knife-points of HCl-phenylhydrazin and three of NaAc. If these salts do not dissolve on warming, a little more water is added. The tube is then put in boiling water and allowed to stand from one-half to one hour (this time prevents the mistake with glycuronic acid compounds). The tube is then put in a beaker of cool water and the crystals searched for microscopically. It were better to let the solution cool down more slowly. This method has been severely criticised.

The Neumann method, much recommended by Thierfelder, is as follows: To 5 cc. of urine are added 2 cc. of 50 per cent. acetic acid saturated with NaAc, and then 2 drops of pure phenylhydrazin. This is concentrated by boiling to 3 cc. It is then cooled rapidly. The tube is then warmed and allowed to cool very slowly. If there be 0.02 to 0.05 per cent. of glucose present, in from five to ten minutes the crystals may be seen without admixture of other precipitate. Neumann recommended special graduated test-tubes.

The crystals of phenylglucosazon should be filtered out, dissolved in hot 60 per cent. alcohol, and allowed to recrystallize by adding water and boiling the alcohol away. The purified crystals should then be tested as regards their melting point. If pure, they will melt at from 204° to 205° C.; when impure, from 173° to 194° C.

A very simple method of determining the melting point is as follows (see Fig. 24): A small flask, A, is filled three-quarters full with concentrated sulphuric acid. Through a perforated stopper is inserted a test-tube, B, also one-half full of the same acid. Into this dips a thermometer, C, to which is attached a tube, D, containing the crystals. This tube has a lumen about 1 mm. in diameter and closed at its lower end, and into it are dropped the dried crystals. Very few are required. The tube is attached by a rubber band to the thermometer (of course at a point above the level of the acid). The flask is then warmed slowly with a Bunsen burner and the point noted at which the crystals melt. The precipitate is of yellow needles in sheaves, which are difficultly soluble in water and in hot absolute alcohol, easily soluble in 60 per cent. hot alcohol, and crystallize out if water be added and the alcohol evaporated off. They are insoluble in ether, chloroform, etc., but soluble in glacial acetic acid. Their solution is lævorotatory.

So delicate is this test that, theoretically (if special technic be used), the small amount of glucose of normal urine will give a definite precipitate, seen microscopically, but practically it does not; Zunz has never found definite crystals except in such urines as reduce Fehling's. Theoretically it shows 0.003 per cent. and is too delicate for practical use, and yet we know of one insurance company which refused a man because of glycosuria detected by this test alone. On the other hand, it may fail entirely when the sugar is known to be present. The success depends on the amount of reagents used and on the time allowed to cool. Not all of the glucose is precipitated, the amount of precipitate depending on the concentration of the glucose and the relation between the reagents. From a 5 per cent. glucose solution the maximum precipitation obtained by Fischer was from 85 to 90 per cent. Much depends on the purity of the phenylhydrazin. The preference given to the HCl-phenylhydrazin is that it is crystalline and not a fluid at ordinary room temperature.

The glycuronic acid compounds will give the same test, but the melting point of these crystals is lower,—114° to 115° C. Various sugars give crystals. In pentose is the greatest danger of error with

the other tests, and hence the use of this is most important, since the crystals obtained melt at 159° to 160° C.

Of the sugars, it is given by all of those reducing copper, including also lactose and maltose. Those sugars which differ only in the first

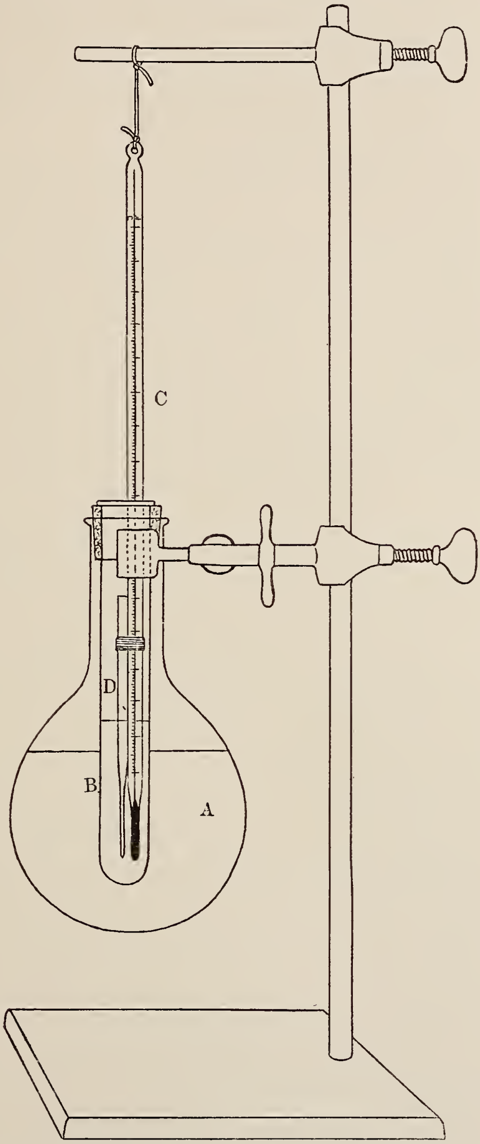


FIG. 24.—Melting point of crystals. A, flask, and B, test-tube of sulphuric acid; C, thermometer; D, fine-bore tube for crystals.

two carbon atoms, with the rest of the formula the same, as, for instance, glucose, fructose, and mannose, give this same osozone. Other

crystals are formed with acetone, hydrazin, oxalic acid, and uric acid. These, however, are not dangerous in human urine, since they do not occur in sufficient amount, and pentose is really the only one to exclude.

With the crystals also is a precipitate of brown scales and oily droplets seen even when a pure solution of glucose is used. This by-product is $C_{12}H_{12}N_2$, and can be washed out with chloroform or 95 per cent. alcohol and then the glucozone recrystallized from 60 per cent. alcohol. Should only brown scales or yellow amorphous precipitate or droplets be found, the test is negative, although glucose may be present.

In review it may be emphasized that there is nothing characteristic in the shape of the crystals; their best solvent is 60 per cent. alcohol; they are best recrystallized by pouring the 60 per cent. alcoholic solution into water and evaporating the alcohol; the hot glacial acetic acid solution, which is soon destroyed, may be tested with the polariscope; that in testing the melting point one cannot expect to get exactly 204° to 205° C., so much depends on the purity of the crystals and on the speed with which the temperature is raised. This should be done as rapidly as possible, since by slow heating the point may be much lowered. This glucosazon differs from galacosazon by the lævorotation of its glacial acetic acid solution, since that of the latter is optically inactive; otherwise they are very similar.

Zunz [64] considers this test one of the most important clinically, and for the further separation of the carbohydrates of the urine we give the table he recommends.

The urine reduces Fehling's.	Gives crystals with phenylhydrazin directly in urine.	Melting point of crystals about 200° C.	Fermentation positive.	Dextrorotatory.	Glucose.
				Lævorotatory.	Levulose.
			Fermentation negative.		Lactose.
		Melting point of crystals about 150° C.	Give orcin reaction.		Pentoses.
			Do not give orcin reaction.		Isomaltose.
	Gives crystals with phenylhydrazin only after the urine has been warmed with dilute sulphuric acid.				Paired glycuronic acid compounds.

The polariscope is very important to use. In the recognition of traces of sugar care must be used, since normally most urines are slightly lævorotatory and some urines are dextrorotatory when sugar is absent (Bornträger in two morphia habitués). Albumin is lævorotatory, hence may cover a slight dextrorotation. The ordinary instrument will detect but about 0.2 per cent. of glucose. The test is of value if the urine be tested before and after fermentation.

Rubner's Test.—This is a modification of the Moore-Heller test. Ten cc. of urine are mixed with an equal amount of PbAc, 1 to 10 solution, and the urine filtered. Ammonia is then added drop by drop until the caseous precipitate just

[64] Jour. Méd. de Bruxelles, July 10, 1902.

remains. The tube is then warmed in a bath at 80° C., according to some, but heated to boiling according to Hoppe-Seyler and Hammarsten. If glucose be present a fine rose-red color results.

Or, to 10 cc. of urine add 3 gms. of the dry PbAc and dissolve by boiling; then filter. To the hot filtrate add NH_4OH and boil hard. It is well to dilute the urine that its specific gravity does not exceed 1010.

If the urine be concentrated it should be diluted one-half with water. Hoppe-Seyler recommends that it be boiled for some time before ammonia is added, and this is to be added to the boiling solution. If it be heated too strongly, however, a non-characteristic brown color appears. If it be warmed only to 80° C. the test indicates glucose, and lactose is excluded. An excess of ammonia ruins the test, hence Voit says to add 0.5 volume of PbAc solution and 0.1 volume of NH_4OH; the urine is then filtered and the filtrate heated. To the hot filtrate is added more ammonia.

Heat Test.—An easy test, sometimes valuable and more delicate than one would imagine and always possible, is the following: One drop of urine is evaporated to dryness in a porcelain dish. It is then warmed gently. A yellowish-brown mass with an odor of caramels is formed at a temperature of 190° to 200° C.

Moore's Test.—Moore's test is one of the first used for sugar. To the urine is added one-fourth volume of KOH or NaOH. On warming is obtained, first, a yellow, then an orange, and finally a dark brown color with an odor of caramels, clearer if the urine be acidified. It may be necessary to boil for some time. It occurs slowly at room temperature. It is the least delicate test. Sometimes a normal urine will darken somewhat, also a urine rich in mucus. The nature of the colored body is not known. The names glucinic acid and melasinic acid have been suggested, one of which, probably CH_3COCH_2OH, will reduce $CuSO_4$ in the cold.

Choice of Method.—Any very positive reduction test indicates sugar. If Nylander's test is only suggestive, it is of value only when we know that the urine did not contain an excess of ammonia, and that it was boiled for some time. The urine may then be polarized, but the presence of a slight amount of sugar may escape this test from the presence of lævorotatory bodies. In all cases albumin must be removed. As a routine reduction test Nylander's is the one to be recommended. Hammarsten recommends that physicians try this first. If negative, no sugar is present. If positive, try fermentation. If this is positive, glucose is the sugar. For the practitioner the fermentation alone is perhaps the best, since it leads to less confusion.

In clearing the urine for further tests it must be remembered that glucose is not precipitated by sugar of lead, but is almost completely by basic lead acetate.

Quantitative Determination of Glucose.—When sugar is known to be present and in good amount, a rough estimate of its amount is possible by the use of Naunyn's table:

2 litres of urine of specific gravity 1028 to 1030 = 2 to 3 per cent.
3 litres of urine of specific gravity 1028 to 1032 = 3 to 5 per cent.
5 litres of urine of specific gravity 1030 to 1035 = 5 to 7 per cent.
6 to 10 litres of urine of specific gravity 1030 to 1042 = 6 to 10 per cent.

In thus estimating the sugar from the *specific gravity*, and in the following calculation, using the coefficient 230, it is assumed that the

change in specific gravity is due alone to the sugar, which is not strictly justifiable, since urea and the chlorides also change somewhat.

Suppose the diabetic urine was 3 litres in amount and of specific gravity 1030: $\frac{2 \times 1.015 + 1.000}{3} = 1.010$, the specific gravity of normal urine if diluted to three litres (on the basis that a normal person voids two litres with a specific gravity of 1015). $1.030 - 1.010 = 0.020$, $0.020 \times 230 = 4.6$ per cent.

In the same way 6 litres at 1030: $\frac{2 \times 1.015 + 4.000}{6} = 1.005$. $1.030 - 1.005 = 0.025$, $0.025 \times 230 = 5.8$ per cent.

Of the common quantitative determinations of clinical chemistry, Fehling's determination is the hardest.

Determination with Fehling's Solution.—The solutions for quantitative work should be made with great care.

SOLUTION A.

Copper sulphate, 34.65 gms.;
Distilled water, q. s. ad 1000 cc.

The $CuSO_4$ should be recrystallized, and on heating at 100° to 110° it should lose 28.87 per cent. of weight; 10 cc. of solution A equal 50 mg. of sugar.

SOLUTION B.

Rochelle salts, 173 gms.;
Sodium hydrate, 125 gms.;
Distilled water, q. s. ad 1000 cc.

The diluted urine is added from a burette to the boiling diluted Fehling's solution until the latter is just decolorized. The amount of urine added $= a$, contains 50 mg. of glucose.

$\frac{50}{a} \times 100 = b$, the percentage of glucose in the diluted urine. Albumin should be removed from the urine.

Procedure.—Ten cc. of each solution, measured carefully with a normal pipette, are mixed in an Erlenmeyer flask of 150 to 200 cc. capacity, and then diluted to about 50 cc. with water. To save time several flasks, four or five, should be prepared in this way at the same time and kept almost at the boiling temperature. The urine should be diluted to contain between 0.5 and 1 per cent. of sugar. The reasons for this dilution are very important; first, 10 cc. of Fehling's copper are reduced quantitatively by 50 mg. of glucose only when the latter solution is at that concentration; and secondly, the urine should be diluted at least five times to rule out the disturbing influence of its

normal constituents. An undiluted urine which contains between 0.5 to 1 per cent. cannot be titrated. Hammarsten suggests to add to it a weighed amount of glucose sufficient to allow of ten times dilution, and from the amount determined subtract that added. Roughly speaking, if the specific gravity be over 1030 it is to be diluted ten times; if under, five times. In careful work, however, one will not be satisfied with this, but will choose the dilution more carefully, so that the percentage shall fall between the above-mentioned limits. The urine is thoroughly mixed with the water (failure to get a perfect mixture leads to some odd results), and put in a burette.

The first flask of Fehling's solution is used to determine the approximate amount of urine to add. To it, on a tripod above the burner and at a boiling temperature, the urine is added from the burette, a cubic centimetre at a time, and the mixture raised to the boiling point after each addition, until the approximate point of end reaction is determined. To the other flasks are then added amounts to fix this point more definitely. For instance, if it be found that the end reaction of the first flask was between 8 and 9 cc., to the second flask is added 8.5 cc. If the fluid was completely decolorized, to the third 8.3 cc. Should this be still blue, to the following is added 8.4 cc., which will decide the point of end reaction. The first flask excepted, to the others is added the urine all at once. It may then be raised to the boiling point, but prolonged boiling is to be avoided. (We notice that some boil two minutes always.)

By end reaction is meant entire disappearance of the blue, but the fluid must not take a yellow tinge, which indicates an excess of sugar (see Moore's Test).

To recognize the end reaction is difficult. Many methods have been proposed, such as filtering the urine, adding calcium chloride, etc., which render the precipitation more rapid. Calcium chloride 10 per cent. is of value, but little should be added. Although the longest, yet the one most to be recommended is, we think, that of studying the color of the clear layer just below the meniscus, that is, the uppermost layer of fluid from which the precipitate first settles. This is found by looking through the flask with the eye on a level with the meniscus and the flask placed in front of a window between which and it is held a piece of white paper. If the eye be in the right position soon is seen a line of clear fluid, the upper layer and involving the whole surface, at the base of the meniscus, the color of which is easily determined. If the eye be placed too low, what will seem to be the line is but the base of the meniscus, hence of very little depth. While watching for this line the flask should not be over the flame, since the currents of fluid with the precipitate will prevent its occurrence. If the line is very slow in forming, it usually indicates that the urine is not

diluted enough or not enough has been added. The reason why the solution cannot be filtered is that the ammonia salts will dissolve the precipitate of $Cu_2(OH)_2$, which is reoxidized by the air to the blue cupric salt. The color of the mass of fluid cannot be determined, since it is full of a red precipitate. It cannot be allowed to settle much since the $Cu_2(OH)_2$ settles upon the glass, and, it being of a yellow color, the color given to the whole is its complementary, that is, blue. Again, much time cannot be allowed for the precipitate to settle, since reoxidation by the air is rapid. We have not obtained satisfactory results by the use of $CaCl_2$, $CaAc_2$, $ZuCl_2$, K_4FeCN_6. The last will itself reduce Fehling's. Hammarsten recommends that after the approximate amount of urine to be added is determined with one flask, to four flasks amounts of urine between these limits and varying by 0.1 cc. be added to each at once, which can then all settle at once, thus saving considerable time. For instance, if the approximate amount were 6.5 to 7 cc., to the four flasks would be added 6.6, 6.7, 6.8, and 6.9 cc. respectively. Thierfelder advises, if the precipitate settles slowly, to rapidly filter a little through a small paper into a flask which rests on white paper. If still blue, this is washed back into the original flask. This allows of some oxidization, and the end results must be confirmed by other flasks.

Other reducing substances may be present, but pentose is the only dangerous one. Since the urine is diluted these are all of little importance.

The method is accurate only if the urine be much diluted, the sugar 0.5 to 1 per cent. Above this point its accuracy is about 0.2 per cent. In case less sugar is present than 0.5 per cent., the urine should be fermented and the test repeated. The difference in the results will be the amount of glucose. Albumin should be removed if more than a mere trace, since this prevents the settling of the precipitate. (See page 171.)

The work of our students has shown the accuracy of this method, thirty-one pairs of students working independently with the same urine and getting results the extremes of which were within the limits of accuracy usually demanded (0.39 per cent.)

Unfortunately, at least half an hour is necessary for one determination, and this is often prohibitory.

If VERY LITTLE SUGAR be present, the urine (1500 cc.) may be precipitated with sugar of lead, then the filtrate precipitated with basic lead acetate and a little ammonia. The precipitate is suspended in alcohol and decomposed with H_2S. The filtrate is then cleared with animal charcoal if necessary and evaporated at low temperature to a small volume. The amount of glucose in this solution is then determined with the polariscope. To exclude lactose and bile acids, both of which would, if present in the urine, be determined as well since they are dextrorotatory, the alcohol is evaporated off, the residue dissolved in water, yeast added, the glucose fermented; the fluid is then filtered, the precipitate being washed with alcohol, the original volume restored and again polarized.

Fehling's solution has been much modified. One of these is *Purdy's fluid*, which is made up as follows:

Cupric sulphate, 4.752 gms.;
Potassium hydrate, 23.50 gms.;
Strong ammonia (U. S. P., sp. gr. 0.9), 350 cc.
Glycerin, 38 cc.;
Distilled water q. s. ad 1000 cc.

Dissolve the $CuSO_4$ and the KOH separately, mix, then when cooled add the NH_4OH; then enough water to make 1 litre. Thirty-five cc. are reduced by 0.020 gm. of glucose. In this case a clear solution is decolorized, since no precipitate is formed, and but two portions are necessary. The test may be performed with the exclusion of air by a simple air-valve, since the dissolved cuprous salts are at once reoxidized, but so long as it keeps boiling this provision is hardly necessary.

Procedure.—To 35 cc. of the Purdy fluid in a 150 to 200 cc. Erlenmeyer flask is added enough water to half fill the flask. The tip of the burette enters through a perforated cork. Through a second perforation a tube bent at right angles allows the steam to escape and keeps air out, also the fumes away from the face. The fluid is then brought to a boil and the urine added drop by drop "until the blue color begins to fade; then, still more slowly, three to five seconds elapsing after each drop, until the blue color completely disappears and leaves the test solution perfectly transparent and colorless." If boiled too long so that too much ammonia is lost, the $Cu_2(OH)_2$ will precipitate. Purdy does not emphasize strongly the need of diluting the urine, and it is less necessary, since his copper solution is so dilute. If the sugar is over 5 per cent., he advises to dilute four times (that is, with three volumes of water).

The urine added ($= a$) contains 0.020 gm. of sugar, hence $\frac{0.020}{a} \times 100 = b$, the percentage of glucose.

For Lehmann's method, see Sahli (1905) and Citron.[65]

Polariscope.—For this very important test and quantitative method the urine must be perfectly clear, so that through the tube of the polariscope when filled even the finest type may be very easily read. All albumin must be removed, since it is lævorotatory. The urine is best cleared, if possible, with Kieselguhr alone. An excess of this is added, the urine well stirred and filtered. The first of the filtrate is to be poured back into the funnel. If, as sometimes happens, this does not clear perfectly, crystals of sugar of lead are to be added and the urine filtered, or these two methods may be combined.

The crystals are somewhat preferable to the solution, which changes the volume of the urine. But some prefer to add 10 cc. of PbAc solution (25 gms. in 100

[65] Deut. med. Wochenschr., No. 44, 1904.

cc.) to 90 cc. urine, and this clears the urine perhaps better. Basic lead acetate cannot be used. Kieselguhr is recommended since lead acetate in any excess alters the physical properties of the fluid and does remove some glucose from urine, although none from a pure glucose solution, and yet Kieselguhr also may remove some sugar. Others recommend a small amount of PbAc and a teaspoonful of Na_2SO_4 (added after the sugar of lead is dissolved).

The tube is then filled, care being taken that no air-bubbles be enclosed, and the angle of rotation measured by the scale on B (Fig. 25), using the vernier, C, for the fractions of a degree.

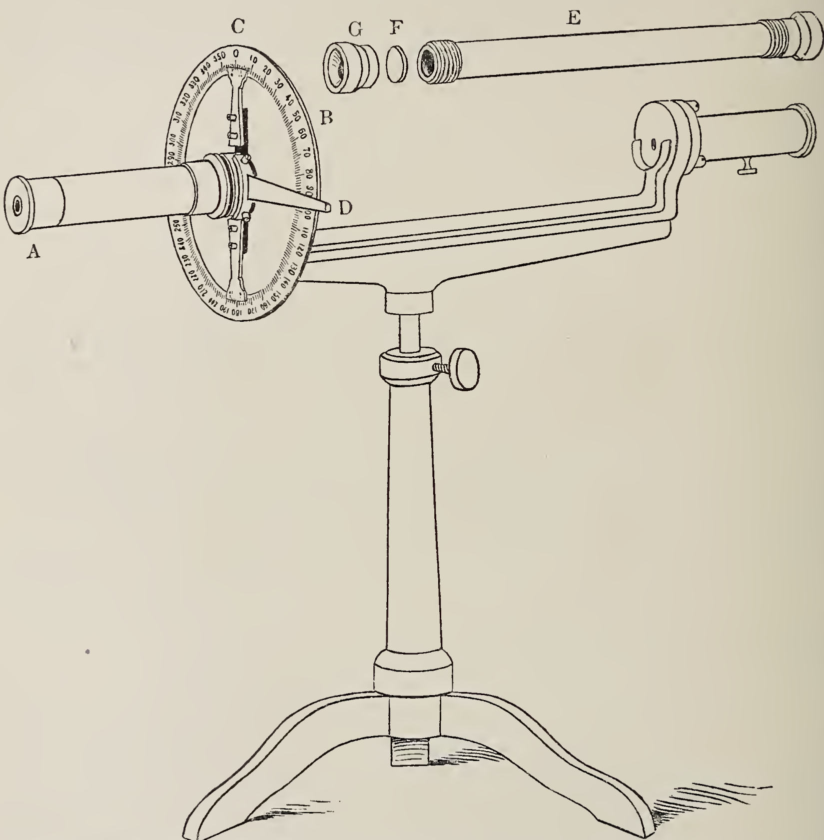


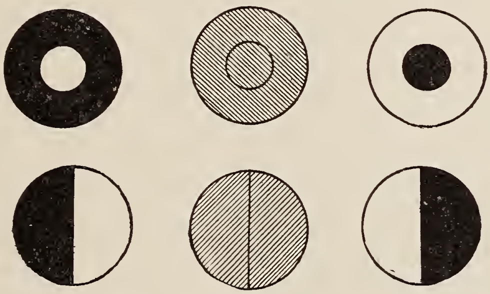
FIG. 25.—Half-shadow saccharometer. A, ocular used in focussing the field; B, graduated disk; C, vernier; D, lever for rotating analyzer; E, tube for urine; F, glass disk; and G, cap for end.

The tube, E, is first cleaned thoroughly and dried. The glass disk, F, of the ends are perfectly clean and clear. One glass is screwed in and then the tube filled with the perfectly clear urine till the meniscus is convex. The second glass disk is then slid on from the side, pushing off the excess of urine and allowing no air to enter, and the metal cap, G, screwed down over this.

The student should understand the instrument that he is using. There are many varieties on the market with slight differences in their construction and greater in their usage. It is seldom, of course, that a real polariscope is used, but

instruments modified for clinical purposes. The polariscope is an instrument which measures the angle of rotation caused by an active substance; the length of the tube is usually 10 cm. or multiples of this, and the reading is in degrees. The specific rotation of glucose is $[\alpha]_D = 52.74°$. If a polariscope be used, therefore, the angle of rotation must be divided by 0.527 to give the percentage of sugar. Those in clinical use are usually "half-shadow instruments," and simplified by using tubes of such length—188.6 mm. (better 189.4 mm.)—that one degree of rotation will equal one per cent. of glucose. Each instrument usually has another tube, one-half as long,—94.3 mm. (better 94.7 mm.),—for highly colored urine. Another instrument which is very popular and much more convenient is the saccharometer, in which the rotation by the sugar is to be balanced by a compensating quartz wedge which is marked with an empirical scale. The great advantage of this instrument is that ordinary white light, as the Welsbach burner, can be used; in the other instrument a sodium flame only.

In using, the field must be first focussed at A and the zero point determined. This changes somewhat with the temperature, particularly in the carelessly used instrument. The tube is then inserted, the field focussed sharply, and the rotation determined. The accuracy with which this can be done will depend upon the clearness of the field which depends on the fluid and focus, the sensitiveness of the instru-



FIGS. 26 and 27.—The fields as seen in the two most common types of clinical saccharometers. The central figures, gray fields with halves of equal illumination, are the zero points. The others are the fields with too little and too much rotation.

ment, and the brightness of the light. To find the end-point with fields of equal illumination there are two common methods. In the one case, the analyzer is rotated until a black band seems to cross the division of the fields. This shadow, purely subjective, is yet of great value. It always appears a little too soon, therefore an average of the readings made from both directions should be used. In the second method the analyzer is slowly turned, always in the same direction, the eye being used but for a few seconds at a time, until the end-point seems to be just reached, that is, where there is no perceptible difference between the two fields. This point will always be attained a little too soon, and in amount equal to the sensitiveness of the instrument. Hence, also, several readings should be made, turning from both directions, and an average taken. In all cases it should be remembered that

the eye should be used but for a few seconds at a time, not over fifteen, to prevent fatiguing the retina. The depth of illumination of the whole field should be judged, and not of contiguous portions.

The half-shadow instruments, modifications of the Laurent, have been constructed to give readings which are sensitive to a very high degree, of even 0.02°, but it should be remembered that the principle on which the instrument rests is itself inaccurate to about 0.2 per cent., hence one gains very little from these very minute readings and some very careful work has only the appearance of accuracy.

The ends of the tube must be planed at a right angle to its long axis. If their axis forms an angle of over ten degrees its use is impossible. This is easily recognized by putting a tube in the instrument, focussing carefully, and then revolving the tube. The same effect is produced as if the analyzer were rotated. Leather washers are necessary to prevent too much tension of the metal cap upon the glass disk covering the end. If this glass is subjected to too high tension, it becomes doubly refractive and a similar error arises as the above, hence before any readings are made the tube should be rolled between the fingers. If this causes the two fields to change in relative intensity, it may indicate one of the two above mentioned errors. If the whole of the field is not equally sharp the solution is not homogeneous or the tube dirty. This is also recognized by rolling the tube.

The normal urine is slightly lævorotatory (0.005° to 0.18°). A trace of sugar may therefore be hidden by a rotation of practically zero. In some cases the urine is dextrorotatory when glucose is not present. Such were two cases of morphia habit (Börntrager). A polariscope is an instrument for the laboratory; for the practical man it is a great (though expensive) aid in quantitative work. Albumin must be removed, it being lævorotatory. Should the worker make up a glucose solution and test it with the polariscope, he must remember to use a solution which has stood for at least a day, since glucose shows a birotation when first dissolved.

Fermentation: Specific Gravity Method of Roberts.[66]—The amount of sugar is estimated from the difference in specific gravity before and after fermentation. The urine should be acidified, if necessary, with tartaric acid. The specific gravity is first carefully determined, using a very accurate aërometer and paying due regard to the temperature of the fluid. A piece of washed yeast the size of a hazel-nut is then added and the urine allowed to ferment at from 15° to 35°C. (a temperature of 34°C. is the best) until it gives no further qualitative test for sugar. This takes from twenty-four to forty-eight hours. The sediment is brought into suspension and the specific gravity again tested. The difference in the specific gravity multiplied by 234 gives the percentage of sugar. It were better that the pycnometer method of determining specific gravity be used, since the aërometrical method, at the best poor, is hardly delicate enough for this work. With accurate work the results are correct to 0.1 per cent.

[66] Edinb. Med. Jour., October, 1861, p. 326.

Albumin need not be removed. The sugar should be at least 0.5 per cent. A greater accuracy is therefore claimed for this than for Fehling's method.

Fermentation: Gas-Volumetric Method.—The Einhorn method of determining the amount of sugar by the amount of carbon dioxide produced is thought by many to be a failure, since the amount of gas produced depends on the amount of yeast, its activity, the temperature, and many other factors; yet it is possible to control well all of these factors, and we have seen excellent results with it. Lohnstein's [67] instrument is said to be accurate and to give the result in six hours using undiluted urines.

The Roberts method is the best for the practitioner who has not a polariscope nor the time for the titration. The factor used in determining the per cent. will be found to vary in various text-books, but the one to use will depend on the method employed, each modification necessitating a different coefficient. Unless one is careful in the details, an error as high as 5 per cent. of the total amount may be made.

Levulose is a sugar widely spread in the vegetable kingdom, particularly among the fruits. It is often present in the urine of diabetics, but associated with glucose. There are, however, a few cases of pure lævulosuria on record (Naunyn) in which this sugar was present in about 1 to 2 per cent; but, as a rule, there is less than 1 per cent. Rosin and Laband reported recently [68] an interesting case of pure lævulosuria (about 0.6 per cent., with considerable lævulosæmia, even 0.5 per cent. when the urine was negative), and uninfluenced by the ingestion of even 100 gms. of levulose or glucose. It is interesting that it is excreted, since levulose is the sugar most easily used by some diabetics, but by no means by all. If large doses are given, it is excreted almost entirely as glucose. In non-diabetic persons may occur spontaneous lævulosuria.

Most of the observations of lævulosuria are in doubt, since other lævorotatory bodies may have been thus interpreted. It is to be suspected when the percentage of sugar determined by polarization is less than that by titration and the lævorotatory body is fermentable. In sixteen cases Rosin and Laband found a lævorotation (titration minus polarization) of 0.3 to 1.7 per cent. (as glucose). No acetone present.

Levulose includes among others laiose and fructose. Fructose gives reactions very similar to those of glucose; reduces copper somewhat less readily (10 per cent.), ferments, and has an angle of lævorotation of uncertain amount. Its characteristic test is that of Seliwanoff, which it is well to use in all cases of diabetes, yet Rosin, also Fr.

[67] Münch. med. Wochenschr., 1899, No. 50.
[68] Zeitschr. f. klin. Med., 1902, vol. xlvii. p. 182.

Müller,[69] warn against the test, stating that it must be confirmed, since glucosamin gives the same. A moderately dilute hydrochloric acid (1 volume of HCl to 2 volumes of H_2O) solution of resorcin is warmed and a little levulose added; the fluid at once becomes a beautiful red, due to a precipitate which is soluble in alcohol to a fine red color. Levulose, if warmed with a concentrated alcoholic solution of resorcin, gives a brick-red color. It gives the same osazone as glucose. It is a more fragile body than glucose.

The other lævorotatory bodies of the urine which must be excluded are albumin, glycuronic acid compounds, β-oxybutyric acid, and cystin. If the lævorotation disappears on fermenting, the strong probability is for levulose. To be perfectly sure, the sugar must be isolated.[70]

Alimentary Lævulosuria has been much used as a test in the functional diagnosis of liver disease. Strauss[71] found that the ingestion of 100 gms. of levulose was followed by lævulosuria in 90 per cent. (26 of 28 cases) of cases with hepatic trouble, and in but 10 per cent. (6 of 58) of normal men. Ferrannini and Bruining also considered the test valuable. Landsberg[72] could get the test in but 9 of 21 cases (not severe ones), and in four of seven normal persons, hence doubts its value.

Lactose is found in the urine of women during lactation in which case stasis in the lacteal glands is the cause, and of patients who have been long on a milk diet. In feeding experiments it is present after the ingestion of 100 gms. as a rule. In diabetics Voit has found that if lactose be fed they excrete glucose, while in the case of lactating women the reverse is true. The amount present is usually small, but may be as high as 2 to 3 per cent. In the case of lactating women it has been found in the urine of 115 of 148 cases (Ney). Others report it in all cases. It reaches a maximum on the second to the fourth day after delivery.

Lactose is dextrorotatory (52.5°). Lactosazon crystallizes in small yellow prisms arranged in spheres and with a melting point of 200° C. Its reduction tests are like those of glucose, but copper is reduced somewhat less actively, and silver nitrate (ammoniacal) is in the cold. Nylander's test is positive. If solutions of lactose be boiled several hours with dilute mineral acid, it is inverted to galactose and glucose. It does not ferment with ordinary yeasts, though some will ferment it without the production of CO_2. It is to be suspected when the copper and bismuth tests are positive yet somewhat slow, and the fermentation and phenylhydrazin are negative. To detect in the urine

[69] Deutsches Arch. f. klin. Med., 1904, p. 1630.
[70] See Peligot method, Compt.-rend., vol. xc. p. 153.
[71] Deutsch. med. Wochenschr., 1901, Nos. 44 and 45.
[72] Ibid., August 6, 1903.

the fermentation test may deceive. The urine must first be sterilized and pure yeast used, else the bacteria in the yeast and urine will split the lactose, giving a fermentable sugar. If the urine does not ferment, yet reduces copper, lactose or pentose may be suspected. To separate these the phloroglucin test should be used.

Rübner's test is valuable. The urine is boiled with an excess of sugar of lead from three to four minutes, when the solution becomes yellow or brown. To the hot fluid is then added ammonia as long as the precipitate which forms will still dissolve. An intense brick-red fluid is obtained which settles later as a copper-red precipitate with a colorless supernatant fluid. This test is positive if the lactose be from 0.05 to 0.02 per cent. The test is perhaps best performed by adding 3 gms. of PbAc to 10 cc. of urine. The precipitate is then filtered off and the filtrate tested. If the specific gravity of the urine be over 1020 it is best to dilute one-half. If the test be performed as recommended for glucose, that is, the solution warmed but not boiled, no red color is obtained—only a yellow coffee-brown or red, according to concentration. Glucose gives a red solution with a yellow precipitate. Maltose, a little yellow, and levulose, no color at all. To prove that it is lactose present the sugar can be isolated.[73]

Pentoses.—The pentoses, sugars with a chain of five carbon atoms, occur widely in nature, not as such, however, but as products by hydrolysis of more complex carbohydrate molecules, which are very important in the vegetable kingdom. In the herbivora the pentoses play almost the same rôle as the hexoses in man, being glycogen-builders. In the animal kingdom they play an important part as the carbohydrate nucleus in the nucleo-proteid molecule of certain organs, the pancreas, thyroid, thymus, brain, spleen, and liver.

These sugars are found in many, if not in all, normal urines. They were found in beer by v. Jaksch, which perhaps explains their common occurrence in the urine in a beer-drinking country. These sugars, when ingested pure, pass the easiest of all into the urine, and in the case of xylose may be demonstrated even after a dose of 50 mg. Yet not all is excreted. They occur also with glucose in many diabetic urines (Kulz and Vogel, who found them in 64 of 80 cases).

As an independent condition the pentoses were first found in the urine by Salkowski and Jastrowitz in two cases of suspected glycosuria. More recently other cases have been reported, but in 1902 the number had reached only five or possibly six (Brat's case[74]). Bendix later collected twelve cases and adds one. It is interesting that several were old morphia habitués, but the pentosuria continued after the habit was cured in one case, not in another. More recent cases give

[73] See Hofmeister, Lehrb. d. Physiol. Chem., 1899, p. 519.
[74] Münch. med. Wochenschr., 1903.

no such history. It seems to be a chronic condition and without symptoms, the sugar being an accidental discovery. It does not occur in large amounts—for instance, in Salkowski's case, corresponding to 0.07 to 0.15 per cent. xylose, in Bendix's case, 0.4 to 0.6 per cent. Bial [75] found that such cases could assimilate glucose normally, but pentose as well. The only explanation, therefore, that he can offer is that an excess of pentose is formed. Bial also found the assimilation limit for hexose and pentose normal.

These urines reduce copper, but not well, as if only a trace of glucose were present. The reduction does not come at once, but after cooling, and suddenly throughout all the urine; they do not ferment, the urine is but slightly dextrorotatory, and with Nylander's the precipitate is only a gray. It is seen that they resemble the urine of lactosuria. V. Jaksch found that diabetics excreted from 48.98 to 82.02 per cent. of the arabinoses of the food, and non-diabetics 1 to 42.65 per cent. Non-diabetics excrete from 54.8 to 18.7 per cent. of xylose, while diabetics only a trace. Of rhamnose from 63 to 55 per cent. was excreted by non-diabetics, and from 3 to 13 per cent. by diabetics.

Xylose is one of chief general importance. This is dextrorotatory. It forms osazones with a melting point of 159° to 160° C., reduces copper and Nylander's solutions, and gives an orange precipitate with Rübner's test; the furfurol reaction is positive; it does not ferment.

The arabinoses are somewhat different. These are dextrorotatory (104 to 105 per cent.), reduce somewhat better than xylose, form osazones, the melting point of which is from 157° to 158° C. Otherwise they are very similar to the above. The arabinose (i) is of particular interest, since this is the form occurring in the urine.

Tests.—The pentoses give the phloroglucin reaction (as do also lactose and galactose), but with pentose the color test is confirmed by its characteristic spectrum, which is also true of glycuronic acid, which gives exactly the same test. This test, according to Tollen, is as follows: To a few cubic centimetres of urine are added an equal part of HCl (sp. gr. 1.19), then from 25 to 30 mg. of phloroglucin, and the solution warmed until a red color appears. This solution is examined spectroscopically at once for a band in the green. If not present, the solution is brought to a boil and the spectrum again tested.

Salkowski recommends the following modification: Five to six cc. of fuming HCl are warmed and saturated with phloroglucin, leaving some undissolved. This solution is then halved. To the one test-tube is added 0.5 cc. of the suspected urine, and to the other 0.5 cc. of normal urine. Both test-tubes are then placed in a beaker of boiling water. If pentose be present, this tube will soon present a red color, which begins above and extends downward. The solution is exam-

[75] Verh. d. XIX. Kongr. f. inn. Med.

ined spectroscopically. The test-tubes should be removed from the water-bath as soon as the red begins to appear.

To exclude glycuronic acid the osazone must be determined (Salkowski). Two hundred to 500 cc. of urine are placed in a beaker, and 2.5 gms. per 100 cc. of urine of phenylhydrazin dissolved in an excess of acetic acid added (or 3.5 gms. HCl phenylhydrazin with 1.5 parts of NaAc). This fluid is then warmed until boiling begins. It is allowed to stand from one to one and one-fourth hours in boiling water and then cooled. If pentose be present in any considerable amount, a large sediment of crystals will appear. As soon as the crystallization is complete the precipitate is then recrystallized from a hot, very dilute alcoholic solution, and again until the melting point is constant.

The orcin-HCl test is recommended as a more specific one, excluding the glycuronic acid compounds. To the urine is added an equal volume of concentrated HCl, and then a small amount of orcin. The solution is then heated. If pentose or glycuronic acid is present, the fluid becomes a reddish-blue color with a characteristic absorption spectrum. The urine should first be decolorized with animal charcoal. The reddish color may not be seen, or only very transitorily, a green color being obtained. The solution is cooled until only warm, and then shaken out with amyl alcohol. A green fluid with characteristic absorption bands is obtained.

Bial's last modification of the Salkowski-Blumenthal test[76] is as follows: A reagent (HCl 30 per cent., 500 cc.; orcin, 1 gm.; 10 per cent. Fe_2Cl_6, 25 drops) is used. Four or 5 cc. of this reagent are heated to boiling, then removed from the flame and the suspicious urine added drop by drop, but not exceeding 1 cc. A fine green color appears at once, or very soon, if pentose be present. This test, he says, is not given by the glycuronic acid compounds or any other body but pentose. He considers his several critics now answered.

If hexoses be present, these should not be fermented, since the pentoses, although unfermentable, will disappear during the process (attributed to the bacteria with the yeast). These sugars should be precipitated as osazones, and then separated.

Method of Külz and Vogel.—From 1.6 to 3.2 litres of urine are used. For each 100 gms. of glucose are added 200 gms. phenylhydrazin plus 100 gms. glacial acetic acid. The urine is then heated on a water-bath for an hour and a half, cooled, and filtered. The filtrate is again heated on the bath for one and a half hours and filtered. The combined precipitates are well washed with cold water and digested in water at 60° C., which dissolves the pentosazone. Glucosazone is dissolved only on heating to boiling. One litre of water per 100 gms. of sugar is used, and the digestion continued twelve hours. This is repeated fifteen times. The hot extracts are filtered, then allowed to cool, and the pentosazone will separate. This is repurified, using less water, till the melting point is constant.

[76] Deutsch. med. Wochenschr., July 2, 1903.

To separate the pentoses the alcoholic solution is polarized. The zylosazone shows a strong constant lævorotation, while arabinosazone immediately after formation is dextrorotatory and then optically negative.

Cases of pentosuria are exceedingly rare, perhaps because unsuspected and therefore overlooked. This is the only sugar which, with many of the glucose tests, promises much trouble. Suspicious cases of glycosuria, in which the tests are unsatisfactory, should always be further examined.

Inosite.—Inosite occurs in a great many plants and vegetables. It occurs rarely and in small amounts in the urine of nephritics and diabetics, and also in other cases of polyuria. Naunyn mentions a case with 18 to 20 gms. per day, but emphasizes the fact that inosite is not a sugar, and the case was probably diabetes insipidus. Hoppe-Seyler considers that it occurs in all normal urines. The method of isolation and detection is rather long. We give it, since in other conditions also inosite is interesting.

The urine should be evaporated to one-quarter its volume. The urine is then precipitated with baryta water. The precipitate is washed, decomposed with H_2S, and evaporated (albumin must first be removed). After decomposition with H_2S the filtrate is allowed to stand and the uric acid first filtered off. The filtrate is then concentrated to a syrup and treated boiling hot with two to three volumes of alcohol. A precipitate rapidly forms. This is cooled, ether is added, and the crystals will slowly appear. These are to be purified by decolorization and recrystallization.

Tests.—The inosite is evaporated with nitric acid on a platinum-foil to dryness. To the residue are added a little ammonia and one drop of $CaCl_2$. It is then again evaporated to dryness and a fine rose-red residue obtained (Scherer's test). This succeeds only when the inosite is fairly pure.

Seidel's Test.—This is the same as the above except strontium acetate is added instead of $CaCl_2$, and a fine green colored solution with a violet precipitate appears. This test is positive when 0.3 mg. of inosite is used.

Gallois Test.—The inosite solution is evaporated almost to dryness, the residue moistened with a little mercuric nitrate. On drying the solution a yellow residue is obtained, which on high heat is of a fine red color, which disappears on cooling and reappears on warming.

Glycogen (or Erythrodextrin).—This has been found in the urine of diabetics after the sugar disappears or diminishes as a dextrin-like substance which browns on the addition of iodine. The urine reduces copper after long boiling. To detect, the sugar is evaporated to a syrup, and KOH and absolute alcohol added until a cloud due to the potassium salts is obtained. The fluid is then decanted, the precipitate is washed several times with absolute alcohol, dissolved in acetic acid,

and reprecipitated with absolute alcohol. This precipitate is warmed with the alcohol and dried. A white tasteless powder is obtained, soluble in water, which reduces copper slowly and browns on the addition of iodine.

Animal Gum (Landwehr).—This is said to occur normally in the urine. It seems to be a pentose, is slightly dextrorotatory, and not fermentable. With the copper test is obtained a precipitate which on boiling does not blacken. Alfthan found it much increased in diabetes mellitus (1.2 to 36.9 gms. per day, normally 0.1 to 0.2 gm.). It is probably not one, but a group of bodies precipitable by alcohol.

Laiose is a sugar not yet well isolated and the nature of which is uncertain, found by Leo[77] in the urine of some diabetics. It is lævorotatory, non-fermentable, with a salty taste and little reducing ability except after long boiling. It gives an oily compound with phenylhydrazin. It is not always present.

Maltose was present in two cases, but in small and variable amounts.

Isomaltose.—This sugar has been demonstrated in normal urine. Whether preformed or formed from glucose is uncertain, since the latter transformation is very easy. The osazone is in very fine crystals, with a melting point of 150° to 153° C. It does not ferment, or only very slowly, reduces copper and bismuth, and is dextrorotatory. It is to be demonstrated as a benzoate.

Melituria.—In the case of some malingerers it may be necessary to recognize this sugar.[78] In Brown's case the urine was of high specific gravity, gave a positive Fehling's test, but not quite typical, and but very few crystals with phenylhydrazin. It fermented but very slowly, and was dextrorotatory. There may, therefore, have been a trace of glucose present or some of the cane-sugar inverted in the acid urine. The urine may be concentrated, boiled with dilute HCl for from twenty to forty minutes, neutralized with sodium bicarbonate, after which the typical tests of glucose plus levulose (polarization therefore zero) may be obtained.

Acetone.—Acetone occurs in all normal urines, the maximum normal amount being about 10 mg. in twenty-four hours. It is much increased, according to V. Jaksch and others, in the following conditions:

(1) Alimentary. Acetone is increased whenever the carbohydrates of the diet are limited. It is always increased on a rich proteid diet in normal persons, and hence during hunger. It is also produced by a rich fat catabolism, but it requires about 150 gms. to influence the output.

[77] Virchow's Arch., 1887, vol. cvii. p. 108.

[78] Brown, Johns Hopkins Hosp. Bull., May, 1900, p. 101.

(2) Febrile acetonuria. This is the most common pathological occurrence. It may occur with any fever, even light, and has no clinical importance.

(3) Diabetes. In this condition it occurs in the largest amounts, and means an advanced or long-standing case. It usually also means a severe case, yet to this there are exceptions, and its presence need not necessarily mean an unfavorable prognosis. Yet its presence should be watched, as it is some criterion of the acidosis, and both therapy and diet will be in some degree determined by the depth of the test. More than 5 gms. may be excreted daily. Great rises may follow slight fevers; it is decreased by alkalies; it may be reduced by adding carbohydrate to the food, and yet in diabetes its output does not depend alone on the diet, although fat may yield some. In diabetes it tends to increase toward death and in coma. It is present also in the breath, giving it a fruity odor, 150 mg. even being excreted in one hour through the lungs.

(4) Carcinoma cases in which inanition is not yet present. (5) Cases of inanition and cachexia. (6) Psychoses and lesions of the central nervous system, especially those associated with starvation. (7) Autointoxication. (8) Digestive disturbances, especially gastric ulcer. (9) Chloroform narcosis, in which case it is due to the increased proteid catabolism. (10) Pregnancy with a dead fœtus. (11) Certain poisons: *e.g.*, phloridzin. (12) Extirpation of the pancreas.

In most of these cases there is little doubt that its source is proteid. Others claim (Schwarz) that it may also be formed from fats. The immediate mother substance of the acetone is probably diacetic acid.

Acetone, CH_3COCH_3, is a thin, colorless fluid of a specific gravity of .814 (at 0° C.), and a boiling point of 56.5° C., with a quite characteristic odor.

Tests.—The urine must be examined fresh. In almost all acetone tests the distillate of the urine is tested. From 250 to 1000 cc. of urine are used and a little acid, preferably phosphoric, added to prevent foaming. V. Jaksch advises that it be distilled with steam, in which case no acid need be used. A good cooler should be used, especially for quantitative work, although practically this is not necessary. Most of the acetone will pass over in the first 10 to 30 cc. Since diacetic acid is split up to acetone the urines should first be made alkaline and carefully shaken out with ether which is alcohol-free, if it is desired (as it seldom is) to exclude this body. This ether extract may then be shaken out with water and the latter tested for the diacetic acid.

As a preliminary, *Legal's Test* is recommended, and yet this is satisfactory only when large amounts are present. A negative report

should never be made unless the more delicate tests are tried with the distillate. To the urine or distillate, better the former, are added a few drops of fresh concentrated sodium nitro-prusside solution, and then KOH or NaOH until very alkaline. A ruby-red color is obtained which changes rapidly to yellow. The test thus far is also given by creatinin, hence while still red, glacial acetic acid is added in excess, and if acetone be present the red color will change to a purple-red, later a violet, which color-change creatinin does not give, but a yellow changing to green and finally to blue. Paracresol gives a reddish yellow solution; acetic acid a clear rose color. It is given by diacetic acid as well. In place of KOH, Le Noble and Lee used NH_4OH to exclude aldehyde.

Gunning's Test is very satisfactory. To the distillate is added tincture of iodine or Lugol's solution (KI 1.8, I 1.2, H_2O, q. s. ad 30), then ammonia until a deep black precipitate forms, which later grad-

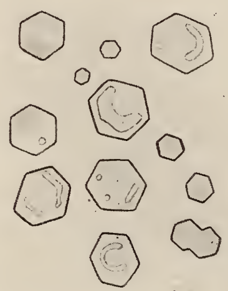


FIG. 28.—Iodoform crystals formed from the distillate of the urine of a case of diabetes.

ually disappears, leaving a yellow sediment of iodoform. This sediment is recognized by its color, odor, and microscopically by the six-sided tablets or stars. The sediment is seldom amorphous. In case of a trace it may be necessary to wait twenty-four hours. If necessary the sediment may be recrystallized from ether. This test may be applied to the urine directly, and is perhaps the safest clinical test for this purpose. Triple phosphate crystals are then also precipitated, and must be recognized. Gunning's test is less delicate than Lieben's, but is given by no other body than acetone. It shows 0.01 mg. per 1 cc.

Lieben's Test.—To the urine or distillate are added a few drops of KOH, and then Lugol's, and the solution warmed. Crystals of iodoform will form. This test will show 0.0001 mg. in twenty-four hours, 0.01 mg. in from two to three minutes. It is the most sensitive but less specific than the above, since alcohol and aldehyde give it.

Quantitative Determination.—The most accurate method is that of Messinger.[79]

An easier and very satisfactory determination may be made as follows:

From 50 to 250 cc. of urine are distilled until the great mass of water is passed over. To the end of the tube, which should have a good cooler, is attached a rubber tube, the end of which dips into water in the receiving flask. A little acid may be added to prevent foaming.

The distillate is poured into a graduated cylinder with a ground glass stopper, an excess (15 to 20 cc.) of NaOH added, and then 20 cc. of Lugol's, which is conveniently made three times the ordinary strength. A heavy black precipitate forms which soon clears, leaving a yellow sediment of iodoform crystals. After standing for some time, ten to fifteen minutes or more, about 40 to 50 cc. of ether are added and the fluid shaken out until the ether contains all of the iodoform. A reading is then made of the volume of ether, 10 cc. are removed in a graduated pipette and evaporated in the air in a weighed glass dish. This is then dried over sulphuric acid and the dish again weighed. The weight of the iodoform multiplied by 0.147 equals the weight of acetone represented in the number of cubic centimetres used. That of the whole ether extract may then be reckoned.

Diacetic Acid. Acetoacetic Acid, CH_3COCH_2COOH.—This body should always be tested for in diabetes. It is the best indication of a severe acidosis. Only a trace, if any, is normal in the urine, and probably none on a mixed diet. It is found only when acetone is present in large amounts, but not always then. Since it is the mother-substance of acetone, its list of occurrences is that of the latter, but it is found much less rarely, since it breaks down easily. In most cases with much diacetic acid oxybutyric acid is probably also present, but the latter is more difficult to determine.

In most cases the cause of its appearance is undernutrition or the failure of absorption. It occurs in certain fevers, especially of children, gastro-intestinal conditions in drunkards, and most important in severe diabetes, especially those somewhat emaciated. Rolleston and Tebbs[80] found it present in large amounts in 33 of 38 cases of gastric ulcer treated either by starvation or by rectal feeding, the test appearing in from two to twelve days, usually one to two days, after treatment begins, and disappearing in from one to fourteen days, usually the fifth, after mouth feeding is begun. Women especially showed the test; age and chronicity of disease seemed of no moment. The same is true of other gastro-intestinal diseases, where it has little importance. In some cases the output of ammonia (index of acids) is as great as in diabetes

[79] See Neubauer and Vogel, p. 760.
[80] Brit. Med. Jour., 1904, vol. ii. p. 114.

(Golla). It may be found in the urine of normal men who have been for some days on a pure proteid diet, and in mental cases with loss of weight and inanition.[81]

Gerhardt's Test.—This very important test is best applied as follows: To 10 to 50 cc. of urine are added a few drops of Fe_2Cl_6 solution, which must not be too acid. This is added as long as a precipitate forms, and then the urine filtered. To the filtrate is added still more Fe_2Cl_6. If diacetic acid be present, the urine takes on a Bordeaux-red color, cherry-red by transmitted, purple-red by reflected, light. The test indicates from 0.4 to 0.5 p.m. of diacetic acid. Cyanates, NaAc, salicylic acid, its allied bodies, salol, aspirin, diuretin, and certain other bodies also will give it. The colors obtained are not just the same, with strong solutions not at all similar, yet by adding various amounts of reagents, exactly the same color, it is said, can be produced, hence the test should always be controlled by boiling the weakly acid urine and repeating the test after the urine has been cooled. The red should be fainter, since the diacetic acid has in part been decomposed, but to boil half an hour will not break it all up. The urine may be acidified with H_2SO_4, shaken out with ether, this with water, and the Fe_2Cl_6 added. A violet-red color is seen in the water layer. The color pales in from twenty-four to forty-eight hours on standing, a necessary part of the reaction to exclude other bodies. The urine should always be tested fresh.

Riegler's test has been much criticised, and its negative nature is unsatisfactory. In its latest modification[82] 15 cc. of urine are mixed with 2 cc. of 10 per cent Jodsäure and 3 cc. of chloroform and shaken. If diacetic acid be present, the chloroform remains colorless, otherwise it takes up a violet color.[83]

β-Oxybutyric Acid, $CH_3CHOHCH_2COOH$.—This is the mother-substance of diacetic acid and hence of acetone. It may, therefore, be looked for when diacetic acid is present in large amounts, but not necessarily found, since it breaks up to diacetic acid and acetone so rapidly. Its list of occurrences is the same for acetone except it is found much more rarely. This acid occurs in the urine of non-diabetics; scurvy, severe infectious diseases, starving insane persons, but these are all in malnutrition. Gerhardt has shown that it is present in the urine of a normal man after some days on a pure proteid diet (about 9 gms. in twenty-four hours were found).[84] This acid is of extreme importance, since to its acid intoxication (a better term is alkali starvation) is attributed the coma. If it once begins, the tendency is for it to increase; often about 50 gms. a day are excreted, and

[81] See also Futcher, Med. News, October 8, 1904.
[82] Zeitschr. f. klin. Med., 1904, Bd. 54.
[83] For criticism, see Voltolini, Zeitschr. f. klin. Med., 1903, Bd. 48, p. 336.
[84] Gerhardt and Schlesinger, Arch. f. exp. Path. u. Pharm., 1898.

in one of Naunyn's cases 100 gms. a day for a long time. Whether oxybutyric acid is toxic alone because of its acid nature or has a specific toxicity is in doubt, the Strassburg school holding the former idea. Wilbur,[85] from animal experiments, injecting the neutralized acid, found the results similar to those of the free acid. He remarks that the alkaline treatment is not as satisfactory in coma as one would expect from theoretical grounds. Larger amounts are reported—even 188 gms. in twenty-four hours (Magnus-Levy); Külz, 225 gms.; and Joslin's case, 437 gms. in three days,—*i.e.*, 3 gms. per 1 K per day.

This acid is lævorotatory,—$[\alpha]_D = -24.12°$. Its presence, therefore, is possible when the percentage of sugar measured with the polariscope is less than that by titration. The presence of other lævorotatory bodies should always be considered, levulose, paired glycuronic acid compounds, albumin, etc.

Detection.—It is probably present if the fermented urine of a diabetic shows some lævorotatory body. It is quite surely present if to the fermented urine be added phosphoric acid and it then extracted with ether and the extract be lævorotatory.

The urine may be fermented, evaporated to a syrup, and an equal amount of concentrated H_2SO_4 added. This is then distilled directly, and crotonic acid is obtained. The distillate cooled gives beautiful crystals with a melting point of 72° C. If these crystals do not readily form the distillate may be shaken out with ether, the ether evaporated, the residue washed with water and allowed to crystallize.

Quantitative Determination.—The methods proposed for determining this acid are several. A good clinical method is the difference between polarization and titration. Both must be accurately done. This is the best method, and in all severe cases is well worth the time.[86] Others, Schwarz, *e.g.*, compare the result with Lohnstern's fermentation saccharometer with the polariscope. An easier method is the polarization of the fermented urine. The lævorotation must remain after the urine has been cleared with basic lead acetate and ammonia. There is no certainty that no acid is destroyed. The method used by Magnus-Levy of determining all of the inorganic acids and all of the bases, and supposing the difference to be due chiefly to this organic acid, must have been exceedingly laborious and does not seem particularly satisfactory.

Bergell[87] recommends the following: From 100 to 300 cc. of urine, fermented if glucose be present, are made weakly alkaline with sodium carbonate and evaporated on a bath to a syrup. This is then cooled and the residue rubbed up with phosphoric acid, cooling it meanwhile, then with 20 to 30 gms. of fused finely pulverized $CuSO_4$ and 20 to 25 gms. of fine sand. This mass is well mixed. The dried mass is then put in a paper sac of the Soxhlet ether extraction apparatus and extracted with ether (which has been freed from water by the fused $CuSO_4$) for one hour. It is then filtered and the $CuSO_4$ washed out with dried ether. The ether is then distilled off, the residue taken up by 20 cc. of water, decolorized with animal charcoal, and polarized.

[85] Jour. Am. Med. Assoc., 1904, No. 17.
[86] See Pavy, Lancet, 1902, pp. 64–143, 207, 347.
[87] Zeitschr. f. phys. Chem., 1901, xxxiii. 310.

The following is the method used in the Strassburg clinic for the isolation of this acid. About 400 cc. of urine are evaporated to about 100 cc. This is roughly saturated with $(NH_4)_2SO_4$, and evaporated to 60 cc. The $(NH_4)_2SO_4$ removes the albumin, some of the coloring matter of the urine, and the concentrated solution of salts is of advantage since oxybutyric acid is much more soluble in water than in ether. When perfectly cool, it is filtered. It is then made strongly acid with H_2SO_4, placed in a litre flask nearly filled with ether and shaken violently for about ten to fifteen minutes. After standing until the urine is settled and the ether solution of the oxybutyric acid is nearly clear, the ether is poured into a separating funnel, and in half an hour or less 20 cc. of distilled water are added, and the fluid shaken violently ten minutes. This will remove some of the H_2SO_4 as well. The water is allowed to separate for thirty minutes. This wash-water is saved for the following ether extractions. The ethereal extract is preserved in a large flask. The original urine is again extracted in the same way and this ether extract washed with the same wash-water, and added to the above. This process is repeated at least five times, the ether extracts after washing with this same wash-water being combined. This wash-water after each ether extract will contain less of the oxybutyric acid, the most going to the ether, and may be finally thrown away. The united ether extracts are then distilled at 60° to 70° C., until all of the ether has passed over. In the residue is the oxybutyric acid. This is transferred to a porcelain dish, the distilling flask washed with ether, and the residue of this when evaporated added to the above. A little distilled water is then added, which causes a slight cloudiness due to hippuric acid. The solution is then allowed to stand at 24° C. It is filtered, using a knife-point of salicylic acid to clarify it, until clear. It is washed with distilled water, and the final volume brought to 25 cc. The angle of polarization is then determined, using a 1- or 2-decimetre tube. In a 1-decimetre tube $[\alpha]_D = -24.16°$. The rotation determined by this will give the percentage of acid in the original amount of urine.

Ten cc. of the solution used in polarization may be titrated with fifth-normal NaOH, using phenolphthalein as indicator. The number of cubic centimetres necessary multiplied by 0.104 equals the number of grammes of oxybutyric acid in 10 cc.

Darmstadter[88] proposes the following method: To 100 cc. of urine is added enough soda to make it weakly alkaline. It is then evaporated on the water-bath almost to dryness, and the residue is washed by 150 to 200 cc. of 50 to 55 per cent. H_2SO_4 into a 1-litre flask. This flask is then connected with the cooler of a distilling apparatus. Through the cork of the flask also enters a dropping funnel. The flask is just heated by a small flame until foaming ceases, then strongly, water added little by little from the funnel as it distils away, until 300 to 350 cc. of distillate have collected. This will take from two to two and one-half hours. The distillate is shaken out two to three times with ether, the ether residue heated a few minutes on a sand-bath at 160° C. to drive off the fatty acids, dissolved in 50 cc. of water, filtered, and the watery extract of crotonic acid titrated with tenth-normal NaOH, using phenolphthalein as indicator. One hundred cc. tenth-normal NaOH = 0.85 gms. crotonic acid. The amount of crotonic acid multiplied by 1.21 equals amount of oxybutyric acid, hence 100 cc. NaOH = 1.0406 gms. oxybutyric acid.

Boekelman and Bouma[89] propose the following simple method:

To 25 cc. of urine in a flask are added 25 cc. of 12 per cent. NaOH, then 25 cc. of benzoylchloride; the flask is corked and shaken hard under cold water for three minutes. The clear fluid is then pipetted off, filtered, and polarized. The benzoylchloride will clear the urine of carbohydrates, albumin, etc., leaving the acid the only lævorotatory body.

Diabetes Mellitus.—The urine in diabetes mellitus is, as a rule, increased in amount. This increase is often not marked unless there is

[88] Zeitschr. f. phys. Chem., xxxvii. p. 355.

[89] Centralb. f. inn. Med., 1902, No. 25.

over 2 to 3 per cent. of sugar, beyond which point it is roughly proportional to the amount of glucose present. In severe cases, that is, with 5 per cent. of sugar or more, there may be from 4 to 5 litres of urine and even 10 litres, whereas one case is on record with 28 litres in twenty-four hours. On the other hand, there are cases with a high percentage of sugar and small amounts; two, for instance, reported by Naunyn, the one with 1400 cc., 9 per cent. of sugar, specific gravity 1040; a second, 2700 cc., 10.5 per cent. of sugar, specific gravity 1047. In other cases the reverse is true, but this is rare except in those cases following injury to the skull, in which during the day the specific gravity may be as low as 1003 with 1 per cent. of sugar (Naunyn's case); also in beginning chronic interstitial nephritis, and when the patient is very weak.

The urine has a high *specific gravity*, this being a condition with a high specific gravity and an increased amount of urine, but the specific gravity bears little relation to the latter. It is usually 1030 to 1040. Naunyn's maximum was 1060, and he mentions a case reported with 1074. With a specific gravity of 1030 there is almost always a diuresis.

The sugar is glucose, yet levulose and pentose and other carbohydrates are also present; in rare cases levulose alone. There is an increase also of the *unfermentable carbohydrates* (a minimum of 20 gms. instead of 1.6 gms.; normal maximum, 5 gms. per day).[90]

The urine is of a suggestive pale greenish-yellow color. It will ferment spontaneously, the fermentation resulting in the evolution of CO_2 and a sediment. This fermentation may occur in the bladder and a sugar-free urine be excreted; again, the fermentation may give no gas.

In testing the urine qualitatively for *glucose* it is important to choose the right specimen. In a severe case with sugar present at all hours this is not important, but in those mild cases with a very slight output, and for only a few hours after a rich carbohydrate meal, the sugar solution may in the whole twenty-four hours' specimen be so dilute that it escapes notice, while if the urine voided from two to four hours after a heavy carbohydrate meal be examined the test is clearly positive. It is well, therefore, to recommend to a suspected case a carbohydrate meal, preferably a breakfast, and examine the urine voided four to six hours later. It has been found that the maximum excretion is in the late forenoon, even when the ingestion of carbohydrates extends over the whole day. There is another maximum, somewhat less, in the late afternoon. Naunyn teaches that cases must be separated according to the percentage of the sugar excretion, the "intensity," and to the total amount of sugar excreted in a day,

[90] Edsall, Am. Jour. Med. Sci., 1901.

the "size." Cases can be compared in this way only when on a constant diet.

In cases with excretion continuous a minimum occurs late at night and early in the morning, a maximum late in the forenoon and at about 6 P.M. In severe cases the variation is little marked and much more may be excreted during the night than during the day (note resemblance to the excretion of water and solids in nephritis). In the light cases the urine may be sugar-free during the night and even reach 3 per cent. during the day. Some cases will be sugar-free for months and then have periods of glycosuria. It is thus seen that the mild cases may easily be overlooked in case but one specimen is examined. The output is greater in hot than in cold weather, true also of the carbohydrate of normal urines.

The amount of sugar per day is often 800 gms., and in one case 1500 gms. in twenty-four hours. Such outputs occur only when the patient is on a liberal diet. On a strictly proteid diet cases excrete seldom over 100 gms., very rarely 200 gms.

As regards the relation between water and glucose excretion, diabetics respond to an increased intake more slowly than do normal people. In general it may be said that the water excretion depends upon the glucose, although this is not true of the day and night variations.

Influence of Diet.—The output is increased by a carbohydrate diet, especially dextrose and the polysaccharides of this. It is less influenced by levulose, lactose, and their polysaccharides. The starches of potato and oatmeal seem very well borne. If levulose, for instance, be fed, the sugar excreted is glucose, and yet a diabetic stands levulose quite well for a day or two, not longer. Lactose and cane-sugar are similarly treated. Fat causes no increase. Proteid causes a slight increase. In severe cases the output also varies with gastro-intestinal troubles, which are so common in diabetics and which may prevent absorption of sugar. Muscle work decreases it, and psychical influences increase it much or bring a latent case to light, such as fright, mental strain, or worry. Hence in diabetics a peaceful mind is one of the essentials. Mendel and Lusk [91] found on a constant proteid-fat diet the constant ratio of glucose to nitrogen of 3.65 : 1, that is, the same as in the phloridzin diabetes of dogs.

They recommend this as a method of prognosis. The patient is put on a meat-fat diet (rich cream, meat, butter, and eggs), and the twenty-four hour urine of the second day is collected (the day ending to include the early morning urine). If N : glucose : : 1 : 3.65, it means the complete intolerance for carbohydrates and probably a quickly fatal outcome.

Intensity of Glycosuria.—On a rich carbohydrate diet the percent-

[91] Deutsches Arch. f. klin. Med., 1904, vol. lxxxi.

age rarely exceeds 6 to 8 per cent. Naunyn mentions a case of 11 per cent., while others a case of 20 per cent.

The effect of *acute infections* is particularly interesting, since in pneumonia, for instance, there is a remarkable diminution in sugar output with even an increased tolerance to carbohydrates, hence not due to the diet, this effect beginning with the rise of temperature. The explanation is not known. On the other hand, the sugar may be increased, or in a case during an intermission be present, only during the fever, hence the statement that the complicating disease has been the cause of the diabetes.

Chronic diseases tend to diminish the sugar output; for instance tuberculosis of the lungs, nervous diseases, circulatory disturbances with albuminuria, and nephritis. In some cases the glycosuria wholly disappears, and hence cases are reported as cured, especially claimed for Bright's disease. This is not due alone to the diet, since the tolerance to carbohydrates is also increased. Neither is it due to the kidneys, since the percentage in the blood does not increase. It may be due to the cachexia sometimes present.

The sugar output is subject to spontaneous fluctuations of considerable amount, even 100 per cent. These cannot be explained in any way except as variations in tolerance.

Severity and Tolerance.—A light case is, according to Naunyn, one which can eat daily 60 gms. of bread and remain sugar-free for a long time. By "paroxysmal tolerance" he means one that can stand considerable carbohydrate and be almost sugar-free, and yet which it is impossible to rid of that last trace. These are severer cases. The old rule that a light case was one which was sugar-free on a strict diet will not hold, since even a proteid diet is not sugar-free and severe cases may keep sugar-free for some time, and yet it be expensive for the body. There is a constant tendency for a large glycosuria to increase, and the greater the glucose output the weaker becomes the tolerance. The slight glycosurias tend to diminish. This tolerance suffers more from large amounts of glucose at one time than from the same amount in divided portions. The reverse is also true that tolerance is increased by a brief period of very slight excretion more than by a longer period of moderate, hence in treating a diabetic the value of the "hunger day," during which by a total fast the patient is sugar-free for twenty-four hours, and on the following day he will often be able to stand without any glycosuria an amount of bread which previously would have caused a marked rise.

A case of transitory "diabetes" with acidosis is reported by Mann [92] which lasted sixteen days, then disappeared even though much sugar was consumed.

[92] Berl. klin. Wochenschr., 1904, No. 30.

Coma and Acidosis.—By "acidosis" Naunyn meant the production in the body by disturbed metabolism of acids in abnormal amounts sufficient sometimes to cause an acid intoxication, or, better expressed, an alkali starvation, resulting, it is believed, in the diabetic coma. The urinary symptoms of acidosis are the appearance of large amounts of acetone or diacetic acid, and, in severe cases, oxybutyric acid, probably the mother-substance of the others. The production of these bodies is not characteristic of diabetic disturbances of metabolism, since a normal person on a sugar-free diet will in a few days produce them all. In diabetics there may be from 20 to 30 gms. of oxybutyric acid excreted daily for years. When an acidosis once begins the tendency is for it to increase. It is increased also by a rigid diet; when the strict dietary treatment of diabetes was first practised the clinicians were surprised at the number of cases of coma, which resulted since the already partially poisoned organism was further burdened by an increase due to diet; hence the value of making the patients sugar-free by rapid reduction rather than a reduction in carbohydrate extending over a long period of time. As coma comes on there is usually a sudden rise in these acid bodies, coma being preceded by days or even months with a daily output of 20 gms. or more of oxybutyric acid, but the output of 25 gms. indicates oncoming coma (Herter). The greatest increase follows the improvement in coma, for it is not the acid in the urine which causes the trouble, but the acid which has not been excreted. The presence of acidosis means usually a severe case, or at least an advanced case in which emaciation has begun, and yet the condition may exist for years. Patients with much acidosis die of coma unless from some intercurrent disease, and there is no case of true coma yet studied without a previous acidosis. The amount of these bodies is perhaps better estimated by the ammonia output than by their direct determination, since the symptoms are caused by a withdrawal of the body alkali which the ammonia protects. An increase of ammonia means the presence of at least 10 gms. of oxybutyric acid per day,—a marked increase, about 15 gms., 4 gms. of NH_3 indicating 16 gms. of the acid (Herter). Naunyn considers that over 3 gms. of ammonia per day means danger of coma, and that if 4 gms. per day are excreted coma is sure to result unless alkaline treatment is begun at once, and then the end is only delayed. Coma was the terminal event of 18 of Naunyn's 44 fatal cases, the most of them young persons from twenty-one to thirty years of age.

A sign, sometimes important, of coma, seen well in many cases and in one of ours, is the appearance of large numbers of granular casts giving a gross sediment. This may appear with the coma or give warning twenty-four hours in advance (Külz sign, page 266).

Among the other urinary symptoms in diabetes is an increase in

the outputs of the creatinin (even 2 gms. q. d.), uric acid, phosphoric acid, and sulphuric acid. In all of these conditions, however, the increase is due to the diet and not to the disease. Oxalic acid is also increased, especially as the sugar disappears (even 1.2 gms. per day). The animal gum of Landwehr is much increased.

Albumin is quite often present. In Naunyn's cases of pure diabetes 32 of 94 had albuminuria, 17 of whom showed occasional traces, 6 long-standing traces, and 10 much albumin. Ruling out those cases in which the albuminuria is due to complicating diseases, Naunyn considers that there is a certain relation between diabetes and albuminuria resulting from the effect of glycosuria on the kidney. On the other hand, it is interesting that as a case of chronic nephritis develops, the glycosuria gradually disappears, and hence cases of the so-called "cures." In some cases the glycosuria and albuminuria may alternate.

Diabetes Insipidus.—The cases from this clinic were recently reported by Futcher in the Johns Hopkins Hospital Reports.

This is a very rare condition, Futcher reporting from this clinic but four cases, or 0.001 per cent. of admissions. It occurred particularly in young men. Jacobi considers, however, that fully 25 per cent. of the cases are in children under ten years of age. The cases may be divided as *primary* or *idiopathic*, which cases have no known lesion, and the *secondary* or *symptomatic*. Such cases are the result particularly of tumors of the base of the brain, trauma or hemorrhage, lues of the brain, and basilar meningitis. It is also symptomatic of certain diseases of the abdominal viscera and of the spinal cord. There is a polyuria sometimes present in the psychoses, particularly hysteria, epilepsy, and chorea, which in some cases would suggest diabetes insipidus.

Polyuria is the only necessary symptom of the disease. From 20 to 40 litres may be voided in twenty-four hours, and in one case, 43 litres. In two cases of children the amount voided daily was almost equal to the body weight. The urine is pale, watery in color, faintly acid in reaction, and of exceedingly low specific gravity—from 1000 to 1005. (It is exceedingly important in this disease that the temperature correction in the specific gravity determination be carefully made.) In other cases there may be a polyuria with a normal specific gravity; *e.g.*, 6 litres and 1017. Albuminuria and cylindruria are absent, this absence indeed being the important point to rule out chronic interstitial nephritis.[93] Sugar is also absent, and yet there are cases of diabetes insipidus which will develop into diabetes mellitus, and *vice versa*. Brackett's case[94] began with sudden onset as polyuria following mental shock, and just before death, seven months later, the

[93] See Blaikie's case, Lancet, 1904.
[94] Lancet, 1899, No. 25.

specific gravity rose from 1002 to 1006 to 1026 and much sugar appeared. One interesting thing which has attracted considerable attention is the fact that certain patients will void an amount of urine in excess of the water ingested. For instance, in one of Futcher's cases, very carefully studied both as regards the measuring of the fluids ingested and that of the urine, and in which the patient was carefully watched to avoid any errors, the urine exceeded the intake of fluids, even including the water content of the solid food, by an amount varying from 400 to 6355 cc. per day. And this continued for a long time.

The urea may reach 80 gms. per day or more. This is due to the diet, such cases having an enormous appetite; in other cases it is diminished. The sodium chloride and phosphoric acid output is normal, sometimes slightly increased.

Inosite is occasionally found. This occurs in normal persons with polyuria resulting in the drinking of large amounts of water, and hence has no importance, it probably being the inosite washed out from the muscles. The total carbohydrates are not increased (Edsall, Alfthan).

Many believe that a condition of bradyuria exists, that is, that the fluid ingested causes an increased urine output more slowly and the increase lasts longer than normal.[95]

Glycuronic Acid, $CHO(CHOH)_4COOH$.—Glycuronic acid is an intermediate product of glucose metabolism, and appears only when it is saved from further oxidation by union with some body with which it can be conjugated, as camphor, or substances arising in the body as indoxyl, skatoxyl, paracresol, phenol, et al., or certain nitrogenous combinations, as uramidoglycuronic acid. It occurs in amounts less than 25 mg. per 100 cc., but its amount varies with these other bodies, and hence its variations are accidental so far as it itself is concerned. The acid crystallizes with phenylhydrazine in beautiful needles whose melting point is 114° to 115° C., but free acid does not occur in the urine. It has the reducing properties of glucose for Cu, Bi, and Ag, reducing copper as well as glucose. It does not ferment. With HCl and phloroglucin or orcin it gives the same color tests as the pentoses and including the spectrum. It gives the furfurol test. While itself dextrorotary, it occurs always in paired compounds which are all of them lævorotatory. These bodies explain the normal lævorotation of the urine of from 0.05° to 0.17°. It is much increased after the ingestion of camphor which pairs with it, and also after chloral hydrate. The orcin reaction is the most convenient one to use (see page 181).

Its chief clinical importance is the fact that its paired acid compounds occur normally, that they reduce copper after somewhat pro-

[95] Pribrâm, Deutsches Arch. f. klin. Med., 1903.

longed boiling, and are lævorotatory. If, therefore, the sugar reaction is suggestive, the fermentation test negative, and the orcin test good, they may be suspected. Since this acid has been shown to be a product of the normal oxidation of glucose, it is increased in diabetes mellitus, some claiming that in very mild cases the unoxidized sugar is present only in this form. Edsall [96] showed that the excess of benzoyl esters in diabetes was not always due to an excess of glycuronic acid, but found an increase in the unfermentable carbohydrates. They are present in all intoxications. He suggested that their increase may be considered a protective measure of the body to combat these intoxications. Glycuronic acid is increased in a great variety of conditions. Edsall doubts that it can even be used to indicate a latent diabetes, while recently Fisher has given evidence that the pairing of glycuronic acid occurs before any oxidation of the dextrose molecule has occurred, which would rather destroy the theories concerning its importance in diabetes.

Alkaptonuria.—This very rare and interesting condition has of late attracted considerable interest. Its rarity is evident from the fact that but 40 cases (29 of them men) were reported up to 1902 (Garrod). Of these, only four were in America (Futcher), and the number has not much increased since attention was called to the abnormality.

It seems a congenital and life-long condition, although some cases are intermittent, and Mittelbach's patient is confident that his followed an injury.

Since without symptoms, such cases are accidental discoveries, the mother noticing the darkly stained napkins of the infant, or the insurance companies refusing them as diabetics.

Most consider this condition to be a variant of metabolism, the inability of the body to burn homogentisinic acid, hence comparable to glycosuria. Tyrosine while the mother-substance of some of it cannot explain all. It is a normal intermediate product of the breaking down of the albumin of food and of the organs.[97]

Alkaptonuria seems to be a family disease, 19 of 32 cases occurring in seven families, one family having 4 cases, but there is only one case of inheritance (Osler's case).[98] Garrod [99] finds that 60 per cent. of the cases are children of parents who are first cousins. Other views are, an intestinal mycosis, a peculiar intestinal ferment, etc.

The amount of reducing substance excreted varies from about 3.2 to 6.9 gms. in twenty-four hours. It is of interest (said Garrod) that

[96] Univ. of Penn. Med. Bull., April, 1902.

[97] See Langstein and Meyer, Deutsches Arch. f. klin. Med., 1903, vol. lxxviii. p. 161.

[98] Lancet, January 2, 1904; see Futcher, New York Med. Journ., January, 1898.

[99] Ibid., December 13, 1902.

the output is for different cases so constant, and that a person either excretes this amount or none. No traces, no gradual increase or decrease, are seen.

The amount depends somewhat on the diet, being reduced about one-half on hunger days, somewhat by a vegetable diet.

Its period of greatest excretion was supposed to be from one to three hours (Mittelbach) after a heavy meal, a point in favor of its intestinal origin rather than due to abnormal metabolism, since the greatest output of the products of metabolism is from four to eight hours after a meal. Garrod,[100] however, found the latter true here, hence its origin in the tissues.

The urine when fresh is very acid, of normal color, but rapidly becomes dark, reddish-brown and syrupy from oxidation, especially if made alkaline. It gives the copper tests, but not Nylander's, $AgNO_3$ is reduced in the cold, it does not polarize, is not fermented, and gives no crystals with phenylhydrazin.

Of the alkapton bodies, two at least have been isolated, homogentisinic and uroleucinic acids. V. Jaksch includes the glycosuric acid of Marshall, which, however, may for the most part be the first mentioned. Other bodies may be present. Urines supposed to be rich in pyrocatechin are for the most part really cases of alkaptonuria.

Homogentisinic acid, $C_6H_3(OH)_2CH_2COOH$, is the most important and in most cases the only alkapton body. It is to this that the characteristic reactions of the urine are due. Its mother-substance seems to be tyrosine, for this, if fed a patient, is excreted as this acid, especially if the tyrosine be given in small doses.

To *isolate*, the urine is made strongly acid with H_2SO_4 (1 to 12) 75 cc. per 1 litre of urine. It is evaporated on a water-bath to one-tenth volume and shaken out four or five times with three volumes of ether. The ether is then distilled off, the residue dissolved in water (30 to 60 volumes), filtered, the solution heated to boiling and precipitated with 20 per cent. PbAc. This is quickly filtered while hot to separate the resinous brown precipitate; allowed to stand, the lead salts will slowly separate in the cold. These are decomposed by H_2S, the filtrate carefully evaporated first on the water-bath and then in vacuo, and the acid will crystallize out.

Garrod recommends that the urine be heated to boiling, and from 5 to 6 gms. of solid PbAc per 100 cc. of urine be added. When these are dissolved the urine is filtered, and the filtrate allowed to stand twenty-four hours in a cool place. The lead crystals are ground fine, suspended in water, decomposed with H_2S, filtered, evaporated first on the water-bath and then in vacuo to a syrup.

Uroleucinic acid, $C_6H_3(OH)_2C_2H_3OHCOOH$ (?) has also been found in alkaptonuria. It is very similar to the above, with the reactions practically the same. It may be separated from it since it is precipitated by basic lead acetate. Garrod found none in the urine of those in whom years previously others had found it.

[100] Lancet, November 30, 1901.

Baumann's quantitative method:[101] 10 cc. of the urine are mixed in a flask with 10 cc. of 3 per cent. ammonia. To this mixture one adds at once several cubic centimetres of tenth-normal $AgNO_3$, shakes it a little, and allows it to stand five minutes. To the mixture are then added 5 drops of 10 per cent. $CaCl_2$ and 10 drops of ammonium carbonate solution. After shaking, it is filtered. The brown-colored but entirely clear filtrate is tested with silver nitrate. If there at once occurs a strong precipitation of reduced silver, in the second test a larger amount, even twice as much silver solution is added to the mixture of 10 cc. of urine and 10 cc. of ammonia. As soon as one has determined approximately the amount of the silver solution necessary for oxidation, the end reaction may be determined with HCl, it being near when the deep brown fluid on the addition of HCl takes a light red color. The end reaction is reached when the filtrate from the silver precipitate on acidifying with dilute HCl gives a slightly visible cloudiness of AgCl. One can determine this point very sharply after repeating the determination from four to six times. If more than 8 cc. of the silver solution are necessary, on repeating the determination 20 cc. rather than 10 cc. of ammonia are to be used.

One gm. of the water-free homogentisinic acid is reduced with the above technique by a quantity of silver solution which contains 2.6 to 2.65 gms. of silver; that is, 240 to 254 cc. of the tenth-normal silver solution. Hence 1 cc. of the tenth-normal solution indicates 0.004124 gms. of the acid.

The method is rather approximate, having an average error of 6.1 per cent.

PROTEIDS IN THE URINE

Albumin Tests.—For all albumin tests the urine must be as fresh as possible and perfectly clear. This latter may be obtained usually by filtration, and this is the best way, using many thicknesses of paper. If, however, the turbidity is due to bacteria, Kieselguhr is often used. Kieselguhr has its objections, since it quite certainly removes from the urine some of the albumin. MgO is recommended, and also the asbestos filter.

It is always best to dilute a concentrated urine, since all albumin tests are rendered even more distinct. Although the albumin is diluted, this loss is more than offset by the gain from the dilution due to the elimination of the influence of disturbing bodies of concentrated urines.

Hallauer's work[102] is interesting as showing the importance of this. If normal urine be concentrated by heat to one-half its volume and then serum albumin added, the heat and acetic acid test is even improved, but the heat and nitric acid test, Heller's, and the potassium ferrocyanide tests are negative. If the urine be previously evaporated to one-quarter its volume, none of these tests will show the albumin, yet all are positive if this concentrated albuminous urine be diluted to its original volume. The potassium ferrocyanide test is the first to disappear, which it does when the specific gravity is about 1030. Urea and the neutral salts, especially phosphates, are the disturbing bodies.

The specimen should be carefully chosen. In a definite case of nephritis this is not necessary, but in very mild cases the albumin may

[101] Zeitschr. f. Physiol. Chem., 1892, vol. xvi, p. 270, or Hoppe-Seyler, Chemische Analyse, seventh edition, p. 460.

[102] Münch. med. Wochenschr., 1903, p. 1539.

be present only during certain hours of the day. Such patients should send for examination the urine first voided in the morning and that voided at the end of a hard day's work. The morning urine is often albumin-free.

Heat and Acetic Acid.—The albumin is precipitated by heat as coagulated albumin. This method is good unless only minimal traces be present. A test-tube is filled almost full of the clear neutral or faintly acid filtered urine and heated at the top to a boil, the tube being held by its lower end. In case only a very slight trace of albumin be present, it were much better to use an alcohol flame, since the slight deposit on the glass from the burning gas will simulate a very slight cloud of albumin. If a cloud is produced, it can be easily recognized by comparing the upper with the lower half of the tube while holding the tube against a black background. A cloud may be due to albumin or to calcium phosphate and carbonate. A few drops of 5 per cent. acetic acid are then added until the urine is distinctly acid. In case it is albumin, the cloud will rather increase and even become flocculent; with very much present, the tube may be inverted without the loss of a drop. In case it is inorganic phosphates, it will disappear, the carbonates with effervescence. From 1 to 3 drops of 25 per cent. acetic acid per 10 cc. is recommended by Hammarsten. The urine should be boiled after the addition of each drop of the acid.

If no cloud is produced, albumin may still be present, and acetic acid should be added even though the urine be perfectly fresh and remain clear on boiling. A cloud may appear, showing that the urine was not sufficiently acid for the precipitation. Here again the urine should be boiled after each drop. In some cases the fresh urine is too acid for the coagulation, in which case an alkali may be added, which will produce or at least increase the precipitation. If the test appear negative, the test-tube is allowed to stand for about fifteen minutes, time being allowed for the coagulum to appear. In case the urine is poor in salts, the test is much improved by adding one-tenth volume of saturated NaCl. An excessive acid must be avoided, since it gives soluble acid albumin. It is therefore possible to add too little or too much, and a mere trace is easily lost; at least, the faint cloud may be either albumin or phosphates, which, one cannot say, since both will disappear or remain if too much or too little acid be used, and the proper amount to add is hard to determine.

The more chronic the nephritis the whiter the albumin cloud. The more acute the browner the precipitate.

The other coagula which may appear are: The so-called "*nucleo-albumin.*" This is precipitated in the cold by acetic acid. Two tests, the one in the boiled, and the other the unheated urine to which acetic acid has been added, may be compared to see if this body will explain all the precipitate. The urine gives a

better test for nucleo-albumin when diluted; the precipitate is soluble in excess of acid;

Resinous acids, in case a great excess of acetic acid be added. This precipitate is soluble in alcohol; this error should never arise. Such occurs after the ingestion of different resinous bodies,—turpentine, benzoin, various balsams, as copaiba, balsam of Peru, Tolu, also cubebs, etc.

Heat and Nitric Acid.—The urine is boiled as above, and then concentrated HNO_3 added until strongly acid. The albumin precipitate is insoluble in fair excess, and hence the danger here is that too little rather than too much acid will be used. As a rule, about one-twentieth to one-tenth volumes are necessary. Hammarsten recommends one to two drops of 25 per cent. nitric acid per 1 cc. of urine. A flocculent precipitate indicates albumin. The phosphates are dissolved. The urine should be boiled before and after the addition of each drop of acid. If after adding the acid the urine be allowed to cool and a precipitate either appears or increases, it is of " albumose." The " nucleo-albumin" is soluble in this excess of NHO_3. On cooling, uric acid may precipitate, but this should not confuse, since it is granular and colored. If only a trace of albumin be present, the addition of nitric acid may cause no precipitate unless the urine be rich in salts; on the other hand, a great excess of HNO_3 may dissolve the trace which does appear, hence the boiling should be repeated after each drop. Here again the coagulation of a trace may not occur at once, and the tube should be allowed to stand some time, and then the coagulum may be found at the bottom.

By the above technique are excluded the "albumin normally present," the " nucleo-albumin," and " albumose" (Bence-Jones body). The urates may deceive in a concentrated urine, but this precipitate is never flocculent; also the resinous acids. Biliverdin and other pigments are said to confuse, but their precipitate is soluble in alcohol.

Another method much recommended is to render the urine strongly acid with a few drops of acetic acid. One-sixth volume of 30 per cent. NaCl (giving a 4 per cent. solution) is then added. A precipitate may appear in the cold, possibly of globulin, which is increased by heat. The urine is then heated as above. All albumins are precipitated, the " nucleo-albumin" cloud is slight or absent. Albumoses are shown which will dissolve on warming. The resinous acids may deceive. This test is less delicate than the preceding.

The heat and acid tests are very delicate, indicating 0.005 gm. per 100 cc. The test should, however, always be confirmed, since the faintest trace is uncertain; by using them we lose the albumoses.

Heller's Nitric Acid Test.—This is a contact test between urine and nitric acid; if albumin be present there is formed a line of precipitate at the place of contact. It has the advantage that no flame is used. The albumin is precipitated as an acid albumin, which is insoluble in a fair excess of acid. Of all the mineral acids used, nitric acid requires less per molecule of albumin than the others. The pre-

cipitate is, however, soluble in a great excess of the acid. The test is very delicate, indicating, according to some, 0.007 per cent. and to others even 0.002 per cent. (Hammarsten). For this cold contact test is used a very large test-tube, or a wine-glass, or the albumoscope which is much to be recommended (a U-shaped tube with one arm very slender, allowing a beautiful layering of the two fluids [see Fig. 29], called also an horismascope).

Into the test-tube is poured about two inches of urine, and then one-third its volume of concentrated nitric acid is allowed to flow under very slowly, the tube being much inclined; or, the urine is made to flow on top of the nitric acid. Still better, the fluid added last is introduced from a pipette. The nitric acid must be colorless, con-

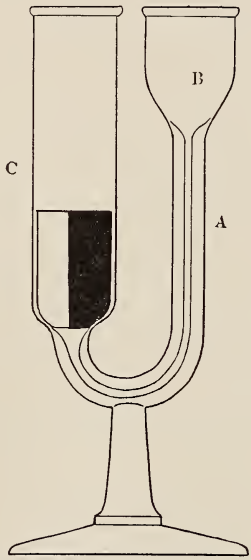


FIG. 29.—Horismascope. A, The arm of the U-shaped tube with fine bore; B, bulb in which HNO_3 is poured after the tubes are filled with urine; C, wide-bore arm for urine, with background.

taining no nitrous acid since its effervescence with urea at the line of contact will disturb the ring and a faint one be lost; the same is true if much carbonate be present, as in an old urine. The line should be searched for against a dark background. In case the ring does not at once appear, one should wait, as it may show later. If no ring appears until three minutes the albumin is less than 0.003 per cent. This ring of acid albumin will appear exactly at the surface of contact; its thinness depends on the amount of albumin and also on the skill with which the urine has been superimposed.

The colored ring which always appears in concentrated urines should not deceive. This may be red or reddish-violet in color, but contains no precipitate.

The urate ring is present in all concentrated urines, and sometimes deceives. It is, however, above the line of separation, and when the test is well made is separated from it by a layer of clear urine from ½ to 1 cm. broad. This ring is broader than the albumin ring, less distinct, disappears on warming, and does not appear if the urine be diluted. To dilute the urine to about one-third volume does not interfere with the delicacy of the test nearly as much as is gained by the elimination of this ring.

One interesting case, the urine of which was sent to the clinical laboratory to demonstrate this fact, shows the value of diluting the urine. A consultant was called to see a case in which the attending physician had made a diagnosis of "albumosuria," the presence of Bence-Jones body in the urine, and had given a hopeless prognosis. It seems that he had tried this albumin test, obtained an abundant precipitate of urates which entirely clouded the urine and which disappeared on warming.

"*Nucleo-albumin.*"—This is present as an opalescent ring 0.5 to 1 cm. above the line of contact, sometimes extending down to it, and which disappears on slightly shaking the tube that the acid may be mixed with the urine, as this body is soluble in nitric acid. In the undiluted urine this ring appears somewhat later, is faint, does not resemble much the albumin ring, and in the case of a diluted urine appears even more rapidly and is clearer than in the concentrated.

Resinous Acids.—These may form a whitish ring above the line of contact and partly clear on warming. These bodies are soluble in ether, which should be added in great excess to prevent an emulsion. The precipitate is pipetted off for this examination. If suspected, the following test should be used. To from 8 to 10 cc. of urine are added 2 to 3 drops of HCl in the cold, which will precipitate these acids. On adding more HCl and heating a red color results. These resinous acids may also be extracted with ether from the urine made strongly acid with acetic acid.

The "albumoses" (Bence-Jones body) will also give a very heavy ring at the line of contact, which disappears on warming.

The bile acids will also give a precipitate in concentrated urines.

Urea nitrate may crystallize out. This line, however, which may form a solid crust between the two fluids, is so solid and so definitely crystalline that it will never deceive.

Hammarsten recommends that as a matter of routine all urines be diluted to a specific gravity of 1005. Dilution excludes all the above disturbing bodies except albumose and nucleo-albumin.

This test should never be trusted alone, but confirmed by another. Many workers recommend that it be used first.

Potassium Ferrocyanide and Acetic Acid.—A few drops of acetic acid are added until the urine is quite acid (containing about 2 per cent. of acetic), K_4FeCN_6 (5 per cent.) is then added drop by drop, avoiding an excess. When the proper amount is added albumin is indicated by a cloud or flaky precipitate. If the observer be expert, this test is more accurate than Heller's. It takes, however, some experience, so much depends on the amount of reagents and the amount of salts present. The test is particularly valuable in quantitative work when it is desired to know if a solution has been rendered albumin-free.

The albumoses are also precipitated. "Nucleo-albumin" is precipitated, but also by acetic acid alone.

A hot urine must not be tested, nor any reagent used containing iron as Kieselguhr,[103] else clouds of inorganic precipitates are produced.

[103] Bardack, Zeitschr. f. inn. Med., 1902, No. 42.

Tanret's Test.—This reagent is made by dissolving 1.35 gms. $HgCl_2$ in as little water as possible with 3.32 gms. KI (hence one molecule $HgCl_2$ equals four molecules KI). To this solution are added 50 cc. of water and then 20 cc. of glacial acetic acid. This solution is added drop by drop to the urine, which will cloud should albumin be present, until the cloud just begins. It is exceedingly delicate. It indicates also "nucleo-albumin," "peptone," soluble on warming, alkaloids, and the albumoses. In French clinics we have seen this test used with the most satisfactory results.

Spiegler's Test.—Spiegler's test consisted originally of $HgCl_2$, 8 gms.; tartaric acid, 4 gms.; glycerin, 20 gms.; water, 200 cc. It has recently been modified by Jolles: $HgCl_2$, 10 gms.; succinic acid, 20 gms.; NaCl, 10 gms.; water, 500 cc.

This is the most delicate test of all. The urine is first filtered, rendered acid by a few drops of acetic acid to hold the carbonates in solution and to precipitate the nucleo-albumin, which is filtered off if present, since this also is precipitated, and then superimposed on the above reagent. A very sharp ring is produced by albumin. By means of it Spiegler claimed 1 : 50,000 could be detected, but as modified it is said there can be detected 1 : 150,000 to 350,000, the latter in the case of Jolles's modification. The advantage claimed for it is that it is definitely positive or negative, not suggestive. This detects also albumoses, but not deutero-albumose. In case the urine be very dilute, that is, specific gravity 1005, the original test is of little value, hence NaCl is an ingredient of the modified solution. That the reagent will not mix with the urine, its specific gravity should be about 1060, hence a heavy acid is used and glycerin or saccharose added, and still more if necessary, as in a case of diabetes.

Various other tests have been proposed: Metaphosphoric acid in solid form, a piece the size of a pea being added to a test-tube of urine; picric acid is used also in the same way. Oliver has recommended papers saturated in the reagents because of the ease in carrying them around. Against all such tests should be said, however, that they are far inferior in delicacy to the above-mentioned tests, and hence fail where most needed.

There is a long list of very delicate tests recommended. In general it may be said that there is danger in these delicate tests, since it is granted a trace of albumin is normally present, and the test used should be only delicate enough to indicate a pathological amount. Almost any two tests which control the one or the other are good enough, providing the worker understand the shortcomings of each and be experienced in their use.

The order of delicacy of these tests is: Spiegler's, Tanret's, then heat and acid; next K_4Fe,CN_6; next Heller's, then picric acid, and various others. This order, given by Huppert, is not accepted by some, who claim that they can with Heller's test properly performed in a wine-glass get more delicate results than with the heat. Senator recommends Heller's test, since it shows albumose. He advised

against heat as the test of preference, since traces are so often lost and albumose may not even be suspected. In general, it should be said that no one test is certain, but each should always be confirmed by at least one other. The heat and acid and the Heller's are a good combination.

Quantitative Determination of Albumin. *Scherer's Method.* —About 500 cc. of faintly acid urine are filtered. About 5 cc. are then poured into a test-tube and this boiled and then filtered until the filtrate be clear. The presence of albumin in the filtrate is tested in the cold with acetic acid and potassium ferrocyanide. If the filtrate be albumin-free the urine is ready for immediate use. In case, however, that a slight trace is still present, two or three drops of 50 per cent. acetic acid are added to the whole amount, well stirred, and a second trial test made. This is to be continued until the filtrate of these sample portions is albumin-free. When near the border-line in some cases one drop of acid too much will spoil the whole and sodium hydroxide solution must be added drop by drop. The work is much facilitated by adding 0.1 volume of saturated NaCl (Cohnheim), this increase in the sodium chloride rendering the precipitation more complete. In this case a clear filtrate is also albumin-free. The addition of this salt is to be recommended as a routine work, and we have saved a great many weary hours by so doing. Of course, a proper correction for change of volume is necessary. The urine now of the right acidity is measured and boiled before the bacteria change this at all. Two portions are then coagulated; first on the water-bath, then over the free flame until the precipitate is flocculent and the supernatant fluid is clear.

The amount of urine used will depend on the amount of albumin present. A general rule is that the weight of dried albumin on a paper shall not exceed 0.3 gm. Larger amounts than this it is exceedingly difficult, we think impossible, to dry to constant weight. In case the urine is very rich in albumin, it is better that a small, accurately measured amount be added drop by drop to a beaker of boiling water or, better, salt solution.

The urine is then filtered through a dried and weighed filter paper, the precipitate washed chlorine-free with hot water, then with alcohol and ether, and then dried to constant weight at 110° C. To obtain constant weight may seem a simple matter, but it is far different, since even at lower temperatures than this constant heating will produce a certain loss in weight. Weighing-glasses with ground-glass stoppers are recommended, and drying ovens in which the heat can be well controlled. The bulb of the thermometer should be on a level with the glasses, which rest on an asbestos sheet. After the weight has become almost constant they should be weighed at stated intervals, preferably

about an hour apart, until the weight is quite constant. Control tests should always be used, and even with the best work the error is often as high as 1 per cent.

Salkowski [104] recommends the following when very much albumin is present. A small accurately measured amount of urine is mixed with 10 to 20 volumes of 95 per cent. alcohol and brought to a boil on the water-bath. It is then cooled, decanted, washed with hot water, filtered, washed, then placed in a weighed platinum crucible, and dried to constant weight at 115° C. The weight of the ash is then subtracted.

Esbach's Tubes.—In these tubes (see Fig. 30) are mixed a definite quantity of urine and a reagent made up of picric acid, 10 gms., citric acid, 20 gms., water, to make 1 litre. Marks on the tubes will indicate the amount of each to be used. The tube is then well corked, and is reversed slowly a dozen times, that the fluids may be well mixed. The tube is then allowed to stand quiet for just twenty-four hours, at the end of which time the height of the column of precipitate is read on the graduated scale of the tube. The reading is given in grammes of albumin per litre.

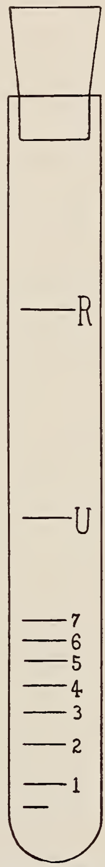


Fig. 30.—Esbach's albuminometer.

It will be seen that this is only a refined method of an older test, in which case the boiled urine was allowed to settle and the height of the precipitate roughly estimated, "50 per cent. of albumin" meaning that the tube was about half full of precipitate. This method has the same objections as the older, and we fear has greater appearance of accuracy than it really possesses.[105]

That the test may be as satisfactory as possible the following points must be observed: The urine must be rendered acid by acetic acid. The tube should not be shaken too vigorously. It should stand in a room of almost constant temperature, since a change in this may easily make a difference of 100 per cent., the precipitate sinking much more rapidly in a warm room. In case a faint cloud appears which does not settle, it is probably "nucleo-albumin," still, traces of albumin will not settle. The test is much more reliable in case the urine be so diluted that the precipitate will rise to a point between the one and the four mark. Sometimes the albumin will collect at the top of the tube, not, however if the urine has been diluted. This test, although very unsatisfactory, is still a favorite of the practitioner. It has its strong points, and gives much information. The two points that should be remembered, however, are, that the temperature must be fairly constant, and that it is not as accurate as it looks; a difference in reading of one or even three grammes per litre in a marked case may not indicate a difference in the urine.

Not nearly enough attention is paid to an approximate estimate from the size of the ring in contact tests, such as *Heller's test*, made

[104] Berl. klin. Wochenschr., March 3, 1902.
[105] Johns Hopkins Hosp. Bull., January, 1903.

in a "Collamore wine-glass" half filled with urine, then underlaid with approximately one-third its volume of nitric acid. By "slightest possible trace" is meant the smallest amount which can be detected as a haze under most favorable conditions (black background, etc.); slightly more means very "slight trace;" a "slight trace" can be seen without a black background and also from above, although the bottom of the glass is distinctly seen; a "large trace" (about 0.1 per cent.) is a clearly seen ring but not flocculent, quite dense but not opaque when seen from above; through the ring made by ⅛ per cent. the bottom of the glass cannot be seen, but a faint ray of light is transmitted; 0.25 per cent. gives a zone quite flocculent from the side and opaque from above; 0.5 per cent. and above, the ring is very dense and flocculent. Above this one cannot go by this method. The width of the ring is not so important (condensed from Ogden, "Clinical Examination of the Urine").

Rössler used Jolles's test in a similar way, but depended more on the thickness of the ring.[106]

Centrifuge Method.—Purdy has recommended that in graduated centrifuge tubes be mixed 10 cc. of filtered urine, 3.5 cc. of 10 per cent. K_4FeCN_6, and 1.5 cc. of acetic acid. The urine is then centrifugalized at a uniform speed of 1500 revolutions per minute in a centrifuge the arm of which is of such length that the distance from the centre of rotation to the tip of the tube is 7¾ inches. It is centrifugalized three times, five minutes each time; $^1/_{10}$ precipitate indicates $^1/_{60}$ per cent. by weight of albumin. In his recent edition he gives a table with the equivalents of the readings. This test, satisfactory as it may seem, has not given very good results in our hands, although better than the Esbach tube. It is an interesting fact that two of the makers of the Purdy centrifuge were unable to supply us with an arm which conformed to his specifications as regards length, hence one must be made to order. It is difficult also to obtain graduated tubes with the sharp point as he represents them. We have found it no easy matter to keep a centrifuge running uniformly at this rate unless one stands over it during the entire time watching the taxometer, and that the time centrifugalized is of great importance.

Roberts and Stolnikow's Method.—This method is based on the observation that with Heller's test a ring appearing from two and one-half to three minutes after the test is made indicates an albuminuria of 0.003 per cent. Diluted urines are therefore tested until a dilution is obtained in which the ring appears in this time. The test should be performed very carefully. The sides of the tube should not be wet with the nitric acid, and the urine should be added slowly from a pipette.

This determines at the same time the "nucleo-albumin" and resinous acids.

[106] Deutsches med. Wochenschr., 1903, No. 19, p. 335.

In the *Lohnstein method* the specific gravity of the urine is determined before and after the albumin is removed by heat and filtration. The difference multiplied by an experimental coefficient gives the albumin per cent.

It is often necessary to *remove albumin* from the urine before continuing with other quantitative work. To do this Hofmeister's method is the best. To the urine an excess (10 cc.) of sodium acetate, 40 per cent. and concentrated Fe_2Cl_6 are added, until the whole is of a red color. The urine is then neutralized or very faintly acidified and then boiled. The precipitate of basic ferric acetate will carry down with it all of the albumin and leave the solution albumin- and iron-free, and this filters beautifully. This method cannot be used if glucose be present, since some ferric oxide remains in solution.

For practical purposes it is sufficient to boil and to add acetic acid until the precipitate is flocculent and the filtrate clear. The filtrate may be further tested as in the quantitative work. The urine should then be restored to the original volume.

Proteids Present.—By ALBUMINURIA is meant the presence in the urine of a coagulable albumin which has escaped through the cortex of the kidney (for false albuminuria, see page 214). Nearly always there are present serum albumin, " serum globulin," and the so-called " nucleo-albumin."

The ALBUMIN QUOTIENT is the amount of serum albumin divided by the amount of " serum globulin" present (Hoffmann). This quotient varies considerably in various cases, and in the same cases during various stages. In some cases serum albumin alone has been found. Such cases were one of cancer of the stomach, and certain cases of nephritis during, however, limited periods. Globulin alone has been found in one case of acute nephritis, in the case of one woman during the puerperium, and in one case of leukæmia. (For " nucleo-albumin," see page 209.)

SERUM ALBUMIN is present normally even to 22 to 78 mg. per 1 litre (Mörner). It is soluble in water, coagulated by heat in acid solution at a temperature varying from 56 to 81° C., depending on the amount of salts present, especially the phosphates also the urea, and lastly on its own concentration; it is coagulated by absolute alcohol, which coagulum is soluble in water unless it has been in contact with the alcohol for a long time. This is rendered even more insoluble by weaker than by stronger alcohol. The solubility of this coagulum should be borne in mind by all doing quantitative work with this precipitate. Serum albumin is lævorotatory $[\alpha]_D = -62.6°$ C. The albumin plus alkali gives a soluble body which, when united with a base, forms an albuminate much less soluble in water than is albumin, and which will therefore give a spontaneous precipitate of albumin in a concentrated urine.

With mineral acids the acid albumin is quite insoluble until a large excess is added; in the case of acetic, however, a very slight over-acidity will dissolve the precipitate.

Serum globulin is a term including several different bodies, among them pseudoglobulin, euglobulin, and fibrinoglobulin of the Hofmeister school, the reactions of which are rather different, but all of which exist in the blood-plasma. Euglobulin and fibrinoglobulin (fibrinogen) are probably always present normally in the urine. They are increased in the mildest forms of albuminuria (the so-called physiological and cyclic cases). In the severer cases albumin is present as well.[107]

The limits of precipitation by a saturated $(NH_4)_2SO_4$ solution are the following, expressed in number of cubic centimetres of the saturated ammonium sulphate solution necessary to add to the urine, the amount of mixture to be in all cases 10 cc. Pseudoglobulin, 3.4 to 4.6; euglobulin, 2.8 to 3.3; fibrinoglobulin, 2.2 to 2.9.

Pseudoglobulin is not precipitated by acetic acid alone. Euglobulin occurs in almost all exudates and transudates, and in many urines, perhaps all. It is precipitated by acetic acid sometimes in the undiluted urine, but usually one must dilute two or three times. The acetic acid must be carefully added, since the precipitate is partly or wholly soluble in excess.

Serum globulin (the group) is present in amounts varying from 8 to 60 per cent. of the total proteid; very rarely only a trace is present. In the blood the ratio to albumin is as 1 : 1.5. Its great increase over the albumin cannot in all cases be explained by its greater diffusibility, since euglobulin, which is constantly present, is less diffusible. The quotient (see page 207) varies in nephritis, the globulin being the variable factor. Oswald considers that an output of euglobulin is the mildest form of albuminuria, and that this is precipitated by acetic acid in the cold. This body is present in largest amounts in parenchymatous lesions. (See also Calvo et al.) As the nephritis improves the relative amount of globulin diminishes, and increases with each acute exacerbation. In cases of contracted kidney and in chronic passive congestion with nephritis the quotient is the higher, from 2.8 to 5.3, but in amyloid disease the quotient may be lower than 1. In acute nephritis it may be very low. It is low but not so markedly so in the albuminuria of pneumonia, but the reverse is true of typhoid fever.

The globulins are insoluble in water, and in the urine are held in solution by the salts. If, therefore, to a beaker of distilled watei a drop or so of urine be added a distinct cloud is seen. They are also

[107] Oswald, Münch. med. Wochenschr., 1904, No. 15.

detected by diluting the urine till the specific gravity be about 1002, then adding one drop of acetic acid.

Test.—The phosphates are precipitated by rendering the urine alkaline with ammonia, and are then filtered off. An equal volume of cool saturated ammonium sulphate is then added to the filtrate, which will perfectly precipitate globulin in neutral solution, the mixture allowed to stand one hour, and filtered. The precipitate is washed with half-saturated ammonium sulphate until the filtrate is albumin-free. Albumose and "nucleo-albumin" are also precipitated. The precipitate of ammonium urate is to be avoided, but this comes later and does not look the same. Serum albumin is not precipitated until total saturation. The precipitate is dissolved in a little water, heated on a water-bath, which coagulates the globulin and fibrinogen and albumose. It is then filtered, the precipitate washed with water and digested on a water-bath with 1 per cent. soda. It is then filtered and neutralized carefully with acetic acid. The precipitate is of globulin and fibrinogen. Albumose would not be precipitated.

To determine globulin quantitatively the filtered urine is rendered neutral with ammonia, and to it is then added an equal volume of saturated ammonium sulphate solution. The mixture, well stirred, is allowed to stand for some hours. It is then filtered through a dried and weighed filter, and the precipitate washed with half-saturated ammonium sulphate until chlorine-free. This filtration is a slow process. The funnel and all are then placed in the thermostat and dried for half an hour at 110° C. The ammonium sulphate is then washed out with hot water, the precipitate dried with alcohol, ether, and then at 110° C. to constant weight. In this case also the amount of urine used should be such that the weight of precipitate could not exceed 0.3 gm.

Euglobulin, Nucleo-Albumin, Mucin, Mörner's Body.—In the urine in a great variety of cases occurs a proteid precipitated in the cold by acetic acid, giving an opalescence or true precipitate especially if a diluted urine be tested. It is difficultly soluble in an excess of acetic acid; the resinous acids should be excluded by the HCl test. With Heller's test the ring is not at the line of separation, but from 0.5 to 1 cm. above it. Both rings, that of albumin and this, may be present, in fact the best "nucleo-albumin" rings are seen in nephritis. It may, however, extend down to the acid. The urate ring should be excluded by testing a diluted urine, which makes the "nucleo-albumin" ring even more distinct. It coagulates at about 56° C., and we have known the diagnosis of albumosuria to be made from this point. The test is always improved if a diluted urine be used, and, in fact, can be obtained in probably every normal urine if the salts be removed by dialysis. It is first seen near the acid, then, as this diffuses upward,

the ring travels upward until all this proteid in the urine has been precipitated and then dissolved.

It is this body which first led to the belief that proteids were normal constituents of the urine. Two other and contrary views were held, one that it was mucus, the other that it was nucleo-albumin, hence the condition was not a true albuminuria; now many believe that it is globulin or a compound of serum albumin, hence is a true abuminuria. A precipitate on the addition of acetic acid occurs in the urine in a great number of conditions, so many that it is hard to classify them, and each writer has done so on the basis of his idea of its nature. Excluding the vesical cases, in which it probably is mucus, it is increased in the new-born; in adults after severe exercise, nephritis, and various acute diseases, especially those affecting the kidneys; fevers, especially pneumonia and typhoid (erysipelas, pleurisy, relapsing fever, meningitis). Its increase in leukæmia, reported first by Fr. Müller, is of interest in connection with the idea that it arose from the nuclei of the leucocytes.

Obermayer found it in 32 cases of jaundice, the amount depending on the intensity of jaundice and ceasing with it. He found it present in scarlet fever in small amounts, diphtheria in the greatest amounts of all, in connection with albuminuria after poisons affecting the kidneys (pyrogallic acid, corrosive sublimate, etc.), in acute yellow atrophy, and after compression of the thorax.

In true nephritis it may precede the true albuminuria and also succeed it, and remain when, by severe dieting, etc., this intermits. Madsen considers it a good test of the earliest irritation of the kidneys. Euglobulin and fibrinogen are said to be the chief proteids in amyloid kidney.

In orthostatic albuminuria this may be the only proteid present, or with albumin and pseudoglobulin. In febrile albuminuria it may exceed in amount the serum albumin. It is present in but traces in chronic interstitial nephritis. When the blood-supply of the kidney of animals is cut off, this body in abundance is excreted, sometimes with albumin, sometimes not, and the same is true in partial suffocation.

Pure mucus is present in the normal urine in traces (4.5 gms. in 260 litres). This may be found in two portions,—an insoluble which gives the nubecula, and a soluble portion precipitated by acetic acid, which is only a very small fraction of the whole. Mucus would be expected since the urinary passages are lined with mucous membrane, and hence the urine will gather a little of its secretion as it passes down to the bladder. This mucin is much increased in catarrhal conditions of the urinary tract, and is added to the urine as a gelatinous precipitate as this passes over the mucosa. It is soluble in ammonia, precipitated by acetic acid, and soluble in excess; from it a reducing

body may be split off. It does not contain nuclein, nor chondroitin, hence it is a mucin, but the absence of the slimy character of the precipitate with acetic acid gives it the term "mucoid." It resembles ovomucoid of the hen's egg (Mörner). In a recent case of prostatitis we found that the acetic acid precipitate was 0.066 gm. per 100 cc. of urine. The urine is precipitated carefully with acetic acid, and repeatedly filtered through a weighed filter till the filtrate is clear. The precipitate is then washed with cold water acidulated with acetic acid, dried, and weighed. Another method giving slightly lower results is the following: A small amount (0.5 gm.) of Kieselguhr is dried at 110° C. to constant weight, mixed with the urine, and dried with the paper and precipitate. It was much more rapid than the preceding. There are, however, other interesting and rare cases of true mucinuria analogous to mucous colitis and fibrinous bronchitis with casts 1 to 10 cm. long and 3 to 4 mm. thick in the urine. Such was v. Jaksch's case of "ureteritis membranacea" in which a spiral cast of the ureter of mucus and fibrin was voided; in Frank's case it was a cast of the pelvis and upper ureter. He named the condition "pyelitis productiva." Four cases are on record. In the above cases the symptoms of the expulsion of the casts resembled those of renal colic.

From the study of many cases of jaundice Obermayer decided that the body was nucleo-albumin, and hence all precipitates with acetic acid were considered nucleoproteid.

Nucleo-albumin also may occur, but it is not the body that usually goes under that name, and it never occurs normally. Its presence has been claimed as due to the breaking down of the cells of the urinary tubules. The kidney is a very rich cellular organ and the disintegration of the cells would be expected to set free a certain amount of nucleo-albumin. Such is true in certain cases of acute nephritis, the one condition where abundant nucleo-albumin may be present; also after poisons which have injured the kidney, and after the circulation of the kidney has been stopped for a short time, this being the first proteid to appear (?). Its origin in cases of jaundice is the bile. Nucleo-albumin is said by some to be present in the blood, and it is possible that a certain amount reaches the urine from this source. It may come from catarrhal conditions of the urinary tract with desquamation of the superficial cells of the mucosa. Such is true in cystitis or pyelitis. In the case of women the genital tract is to be excluded as a source.

It will be seen from the above list that the occurrence of nucleo-albumin is claimed for all cases in which theoretically nucleoproteid could occur; but in some of these conditions a true albuminuria occurs, and in others in which nucleo-albumin should be present in large

amounts (urines containing abundant pus and epithelial cells) it is hard to get any precipitate at all on adding acetic acid.

It is very clear, to one reading reports of cases, that in very few has the crucial test been applied, the proof that it is a phosphorus-containing body which acetic acid precipitated, and positive results would have to be scrutinized carefully, since it would be very easy for phosphorus to be an admixture from the urine.

Again, the "salting out" points with ammonium sulphate do not quite agree with those of true nucleo-albumin from breaking down tissue. Matsumoto gives as limits of its precipitation, minimal 0.1 to 0.8, maximal 1.6 to 2.2.[108]

To prove the body nucleo-albumin it should be insoluble in acetic acid, precipitated by $MgSO_4$, when boiled with dilute mineral acids give off no reducing substance, and on peptic digestion should give nuclein and contain phosphorus, but the last two tests it is almost impossible to apply to the urine.

Mörner's Body.—Work which seemed very convincing and which is now often quoted as that of Mörner.[109] According to him most of the so-called nucleo-albumin is a compound of true serum albumin with an albumin-precipitating body which is formed on the addition of acetic acid. Mörner, by dialyzing large amounts of urine and adding 1 to 2 parts per thousand of acetic acid, and then shaking with chloroform, obtained a precipitate which much resembled nucleo-albumin. This occurs on an average of 41 mg. (22 to 78) per litre of urine. Further investigation showed this to be a precipitate of serum albumin with chondroitin-sulphuric acid, which was always present and the most important, nucleinic acid, which is sometimes present in traces, and taurocholic acid, which is also sometimes present in traces, but which in the case of jaundiced urine may exceed the others in amount. Since there are these three possible combinations the precipitates will differ. If after removing this precipitate a little albumin be added to the urine, a second precipitate results of about 54 mg. per litre, showing that these albumin-precipitating bodies are in excess. Since normally in excess, any increase of precipitate would mean an increased excretion of albumin. The greater the predominance of these precipitationg bodies, however, the more does the precipitate resemble nucleo-albumin. This union probably occurs after the addition of acetic acid. According to the relation between them the precipitate will resemble nucleo-albumin or serum albumin. If the albumin predominates, it may give its own proper tests. These bodies, and their relation, will explain the old statements, based on the common experience, that "a true albuminuria is sometimes preceded

[108] Matsumoto, Deutsches Arch. f. klin. Med., 1903, vol. lxxv. p. 398.
[109] Skand., Arch. f. Phys., vol. vi. p. 332, 1895.

by the excretion of a body precipitated by acetic acid," that "the excretion of mucus may precede or succeed an albuminuria," the belief in a "physiological albuminuria," also the opposing belief of recent years that this so-called physiological albuminuria was merely a nucleo-albuminuria.

Mörner used the following method of isolation:

The salts may be dialyzed out of a large volume of urine and then acetic acid is added, 2 cc. per litre. The precipitate is then dissolved in a little water and again precipitated with acetic acid. It may then be tested for the presence of chondroitin-sulphuric acid by heating on the water-bath with 5 per cent. HCl for a long time. If both sulphuric acid and a reducing body are present, this body is probably present. If the reducing body is demonstrated, but no sulphuric acid, it is probably mucus. If there is no sulphuric acid and no reducing body, and the precipitate then be digested with pepsin and organic phosphorus be found, the nuclein bases may be demonstrated in the products of digestion. Large amounts of urine, however, must be used for its detection.

This explanation of Mörner, satisfactory as it would seem, and evidently based on very careful work, has received little confirmation. Stähelin [110] in one case of jaundice failed to find any of the "albumin-precipitating bodies," and thought the precipitate resembled the globulins, a view held by Fr. Müller in 1885; also in the acetic acid precipitate of the urine of a case of pneumonia with a very heavy precipitate on adding this acid, no phosphorus could be detected. Matsumoto found it chiefly fibrinogen and euglobulin (see page 208). Oswald [111] studied carefully this precipitate with acetic acid in the urines of cyclic albuminurics and nephritics, and decided it to be euglobulin and a trace of fibrinogen. These occur in the blood, but cannot be demonstrated there by the addition of acetic acid, since the salt content is too low.

It is to be noted that in most of the above work small amounts of urine were used, not the large amounts of Mörner; again, that many will not agree that the limits of precipitation with saturated ammonium sulphate are alone sufficient for the recognition of a proteid. In conclusion, it may be stated that, however the present conflict between Mörner and the Hofmeister school may be settled, both agree that there is a constant normal physiological albuminuria.[112]

The Nucleohiston of Lilienfeld is a body arising from the breaking down of leucocytes. It is precipitated by acetic acid and has a high phosphorus content. This is found in the urine especially of leukæmic patients, although its appearance is not alone due to the breaking down of these cells.

Albumin, if present, is first removed; the proteids of the urine are then precipitated with alcohol, the precipitate washed in hot alcohol, then dissolved in

[110] Münch. med. Wochenschr., 1902, p. 1413.
[111] Zeitschr. f. d. gesamt. Biochem., Bd. v, 1904.
[112] See, also, Calvo, Zeitschr. f. klin. Med., 1904, vol. li.

boiling water, cooled, acidified with HCl, let stand, and the uric acid precipitate filtered off. To the filtrate is then added ammonia, the precipitate collected on a small filter, washed with ammonia till the wash-water gives no biuret reaction. The precipitate is then dissolved in acetic acid and tested for histon. This gives the biuret reaction, is coagulated by heat, and this coagulum is soluble in mineral acids.[113]

Fibrinuria.—Fibrinogen, fibrinoglobulin, occurs rarely in any amounts in the urine. The reactions are those of globulin, since this body belongs in that group. To recognize its presence, however, is easy, since there is a spontaneous coagulation on standing.

Excluding those cases in which there is blood in the urine, fibrinuria is rare. It occurs in chyluria and in some rare cases of nephritis. In some cases the urine clots at once after voiding, the clot being sometimes firm and in other cases gelatinous. Or this may occur before voiding, the clots being casts of the pelvis of the kidney or from the bladder (the term fibrinuria, of course, is strictly applicable only to these latter cases). In severe inflammation of the urinary passages, the bladder, ureter, or pelvis of the kidney, these clots may be formed. The reason for this is not known, since most of the inflammatory exudates do not coagulate. We have seen but one good case,—a woman admitted during the last hours of her life with what was evidently chronic parenchymatous nephritis. Only about 5 cc. of urine could be obtained. This was of a rather cloudy yellow color; no blood grossly. After standing for a few minutes it clotted to a solid coagulum. In the decomposing alkaline urine, such as occurs in alkaline catarrh of the urinary passages, masses of pus, mucus, and bacteria may be voided or may even plug the passages, and resemble fibrin casts.

Fragments of tumors have also been found in the urine.

Albuminuria.—Cases of albuminuria may be divided into the false and the true. By the false are meant those in which the urine, as secreted by the kidney cortex is normal, and the albumin is contributed lower in the urinary passages, either as an inflammatory exudate, or lymph, blood, or chyle. By albuminuria in the following paragraphs is meant only the true,—that is, albuminuria due to some disturbance of the renal epithelium, especially of the glomeruli, not of the blood capillaries; the latter are always permeable to albumin, and the great wonder is not that albumin should ever pass through the renal epithelium, but that it does not always. Over all the rest of the body exudates and transudates are always albuminous. In albuminuria occur together serum albumin and "serum globulin." (See page 208.)

Albuminuria without Definite Renal Lesion.—Concerning physiological albuminuria,—that is, the constant presence of a proteid in normal urines,—the pendulum has swung several times. Posner, in

[113] See Kolisch and Burion, Zeit. f. klin. Med., 1896, Bd. 29, p. 374.

1884, first claimed the presence of serum albumin in all normal urines; this was believed in and then doubted, and again accepted on the basis of certain chemical tests for albumin. With the supposed demonstration that these tests indicated rather a mucin or a nucleo-albumin, the physiological albuminuria was again doubted, until recent work, particularly that of Mörner and that stimulated by him, seems to have established beyond doubt the presence of a small amount of serum albumin, or, according to others, euglobulin, in practically all normal urines.

With Spiegler's reagent it is hard to find a normal person with a urine really albumin-free. The absence of true nucleo-albumin may be considered evidence of the physiological nature of the condition, showing the renal cells are not yet injured.

If this is the case, there is no line between physiological and pathological albuminurias except that of amount. By "albuminuria" is now meant a condition in which serum albumin may be detected by the tests accepted in common use as standards, and the cases with small amounts of albumin which pass unnoticed by these tests and require special technique are not included. Hofmeister gives as standard that if Heller's test shows no ring in three minutes the urine is to be considered albumin-free.

Hence the question of albuminuria is similar to that of glycosuria, a very small amount of both bodies being normal, but disregarded unless increased to sufficient amount to give the tests accepted as criteria. The line, however, is an artificial one and very difficult to draw. This gives the teacher considerable difficulty in the medical school, in which the students are taught the very delicate tests, since each year a few discover a positive albumin test in their urine and are rendered very unhappy thereby.

Concerning this proteid of normal urine, the demonstration of which requires very delicate tests or the use of large amounts of urine, see page 209.

The above is the only correct use of the term physiological, although this is wrongly used for cases of albuminuria in the apparently healthy. By albuminuria in the following pages will be meant an amount which can be detected in the test-tube with a few cubic centimetres of urine.

But the so-called FUNCTIONAL ALBUMINURIA is a different matter. The term "functional" Pavy used merely in contrast to "structural," in which case the albuminuria depended on anatomical changes in the kidney. In these cases ordinary tests are used and a small amount of urine, and concerning the presence and nature of the proteid there is no doubt. Senator considers that the albuminuria is truly "physiological" or "functional" when the albumin occurs in small amounts in

young men, is transitory, the further history of that person negative, the urine otherwise normal, and its occurrence following always an unusual and adequate cause, such as severe muscular work or severe exercise by those not used to it, and later, when trained, its absence. But these cases should be placed in a separate group and the term "physiological" used with caution. Such cases are normal men who, after unusual exercise, exertion, exposure to cold, nervous stress, or after unusually large proteid meals, show a temporary albuminuria. According to Senator the cause must be extraordinary for that person, and later, when the organism is used to it, cause no escape of albumin.

Such cases were first described for soldiers, many of the raw recruits showing albumin after a forced march. The figures first given were 16 per cent., then 59 per cent. (Leube). Later examination of the same soldiers shows practically every one albumin-free (Flensburg).

As further illustrations showing that the same may be true also of trained men, are the foot-ball players, in whom Macfarland[114] found in practically every player after a game an albuminuria which lasted for the most part but three to four hours. Müller[115] showed that of bicycle riders after races, eight of eleven showed albumin, and seven of twelve casts of all descriptions and renal epithelium. The urine was normal on the day following. The same is true of athletes, mountain climbers, bicycle riders, foot-ball players, those persons who exercise severely the leg and thigh muscles especially to a degree beyond that to which they are accustomed, and later are able to stand an equal amount without the same result. We may say that it is only a question of limit; practically every one can if he will produce albuminuria, he has only to over-exert himself sufficiently. The most normal man in every sphere of life must still observe certain limitations, and the question comes, Having overstepped these, can the albuminuria which results be termed "physiological"? Of course, the limits for persons differ, and what is physiological for one is pathological for another, but the groups of cases now under consideration concern only those of the highest physical attainments,—trained athletes, young men picked for the army, etc. The fact that Macfarland found of twenty foot-ball players after a game granular casts in the urine of nineteen and blood-casts or corpuscles in six, *i.e.*, the demonstration of every element of acute nephritis, all sorts of casts, hæmaturia, and hæmoglobinuria, is excellent evidence that for a while at least the kidneys were not normal.

In this same group Senator includes cases the relation of which to the normal bounds of the physiological it is more difficult to deter-

[114] New York Med. Rec., 1894, vol. xlvi. p. 769.
[115] Münch. med. Wochenschr., 1896, No. 48.

mine. Among such are albuminurias following violent emotions and an unusually heavy proteid meal. The latter, "alimentary albuminuria," is a form considered doubtful by some, that is, in the sense that the kidney merely excretes an excess of proteid as a part of its normal function to relieve the blood of superfluous constituents, as it does for glucose. Among soldiers, hence men under uniform conditions, Rapp found that 10.7 per cent. showed albuminuria after their mid-day meal. Experiments show that a large amount of certain proteids (for instance, eight or more raw eggs) will in some apparently normal persons, but not all, cause albuminuria; but the amount ingested must be excessive. After much smaller amounts the egg albumin can be detected in the blood-plasma. In nephritis cases small amounts are excreted through the kidneys. The output for normal men begins in about two hours and lasts four.[116] This is confirmed by animal experiments, but the demonstration that not only is egg albumin excreted as such, but serum albumin as well, as shown chemically and by the specific precipitines (it is only just to say that this method [precipitines] has not proved very satisfactory), suggests that the excess of proteid in the blood may have, temporarily at least, placed the kidneys in a pathological condition. An alimentary albuminuria is claimed for the new-born whose intestinal mucosa has not yet developed that impermeability to foreign proteids which later is present, hence the albuminuria when fed on cow's milk.

Prolonged cold baths will cause albuminuria. Rem Picci[117] found from observations on one hundred and fifteen baths of thirty-five healthy men that three minutes at 12° to 13° C., or fifteen minutes at 15° to 20° C. (none at 20°), caused quite regularly a slight transitory albuminuria, minimal in amount, never lasting over twenty-four hours, with casts, and generally diuresis with increased urea and chloride output. This he explained from reflex nervous influences from the skin. It is serum albumin in the urine.

Mental over-exertion is also claimed as a cause in certain cases.

There is special reason for the albumin to appear should several of these predisposing factors occur simultaneously. The intermittent nature of the albuminuria is no criterion, since a truly pathological case may intermit considerably; but in all such cases must be emphasized the appearance of albumin after a very unusual strain or occurrence, and one adequate to explain its appearance; also its very temporary duration. Senator considers that if the amount of albumin exceeds 0.4 to 0.5 gm. per litre it cannot be called "physiological."

[116] For recent articles, see Ascoli, Münch. med. Wochenschr., 1902, No. 10, and Inouye, Deutsches Arch. f. klin. Med., 1902, Bd. 75; and on the opposite side of the question Umber, Berl. klin. Wochenschr., July 14, 1902; for the chemical side, Sollman and Brown, Jour. of Exp. Med., March 17, 1902.

[117] S. J., 273, p. 37, 1901.

Another example of "physiological" albumin is the ALBUMINURIA OF THE NEW-BORN. Often for the first eight or ten days there is a slight amount of albumin with hyaline casts, epithelial cells, and urates present. This is also present in the urine found in the bladder of still-born children, and therefore is not attributable to any changed circulatory or metabolic products after birth. Ribbert gave as an explanation that the kidneys at birth are really not quite "finished," but there still occurs a desquamation of epithelium of the capsules of the glomeruli, hence with the albumin occurs nucleo-albumin from these cells.

The ALBUMINURIA OF WOMEN IN LABOR should be considered as physiological. Some find that in about 39 per cent. of normal cases this is present. It is attributed to the circulatory changes of the kidney due to the work, strain, etc. The condition of the kidney is doubtless pathological, but the cause is physiological, and the albumin usually disappears at once. Little,[118] as the result of very careful work, concludes that albumin is present in the catheterized specimens of urine from about one-half of all pregnant women, being equally frequent in primiparæ and multiparæ. Casts occur with greater relative frequency in multiparæ. During labor these percentages increase, especially in primiparæ. This may be due to the muscular work and increase of blood-pressure during labor. During the puerperium the percentage drops.

"ALBUMINURIA OF ADOLESCENCE" (Gull), "of puberty," "accidental albuminuria," "essential albuminuria," "physiological albuminuria," "Pavy's disease," "cyclic albuminuria of the apparently healthy," "postural," "orthotic," "orthostatic," and "intermittent albuminuria." This group of cases is of far greater importance and interest than the preceding. It is a much larger group than was imagined, since urinary examinations are now more often made on those apparently sound, by insurance companies, army inspectors, and as a result of the neurasthenia resulting from advertisements of medicines. Insurance men say that of the "normal" persons examined while the temperature is above 90° or below 0° F., 5 per cent. show albumin; at other times, about 2 per cent.

This group is of persons enjoying reasonably good health, whose urine either constantly or temporarily contains a trace of albumin. Their number is large, and they certainly can be divided into several groups, which classification it is convenient to use, although the present may strike entirely beyond the bounds of evidence. The above long list of names shows what features may predominate. Posner proposes one quite satisfactory, "essential albuminuria," since the albumin is the only symptom which one can always find.

[118] Amer. Jour. of Obstet., vol. l, No. 3, 1904.

Of the group as a whole it may be said that it includes young persons during adolescence or in the few following years, who are often not of best health, and not robust but anæmic, often children with a neurotic family history and with unstable vasomotor system, who sometimes give in their history such diseases as scarlet fever, diphtheria, et al., which would suggest a latent nephritis, who may continue for years in good health or later show clear signs of Bright's disease. Common to all is the absence of other signs and symptoms of kidney trouble, and if it is cyclic or intermittent the albumin appears in response to ordinary acts of our every-day life, that is, not to an unusual or adequate cause. In some cases it is said to follow walking or other exercise, in others a heavy meal. It is sometimes a family disease, three children in one family showing it (Lacour). In this group are included by some the albuminuria of masturbators and that following sexual excitement. In these cases it may be present only before rising in the morning. Some (Sir Andrew Clark and others) say this proteid is a secretion of the ureter or accessory glands.

A diagnosis is possible only after long careful study of the individual case, including past history and especially the physical signs on the part of the heart and eyes, and even then the autopsy may reverse the diagnosis.

If there be good evidence of past renal disease or any cardiac features suggesting it, the case must be considered one of nephritis. The specific gravity, amount, sediment, etc., of the urine are important in diagnosis. The intermittent nature of the output is no criterion, since this may be seen in true chronic nephritis; nor does the presence or absence of casts help, since hyaline casts may occur whenever albumin does, and careful search shows them in the more truly physiological cases; nor does the typical postural character, for this is seen in cases of acute nephritis after scarlet fever as it recedes (Knöpfelmacher) and in cases of chronic interstitial nephritis and of waxy kidney.

While one case may fall in any one or several of the following groups, we give the classification to emphasize the features which such cases present.

The "ALBUMINURIA OF ADOLESCENCE" is a form separated by Leube from the one great group. It occurs between the ages of fourteen and eighteen years and then disappears. It may be explained by a renal insufficiency relative to the growing organism, the kidney not keeping pace with the physical growth and activity, together with instability of the vasomotor centres. In this group occur most of the cyclic or postural cases, not all, since some of these latter continue to adult life, and not all the cases of this group are truly

postural. The element of heredity is very important. The cases reported by Lommel[119] would fall under this title, since the question of posture was little considered. Of 587 factory workers from fourteen to eighteen years old, 18.9 per cent. showed albuminuria once or many times, in small amounts and for the most part intermittent. Of sediment there was none, or at the most a few hyaline casts and fatty epithelial cells in the centrifugalized specimen. Of 130 patients from the same class, but over twenty-five years old, only one showed albuminuria. Cardiac and vascular disturbances were common. Posner emphasized sexual excesses at puberty as a common cause. Sutherland[120] emphasized the relation between this form and movable kidney present in one-third of his cases, and so common in children.

Cyclic albuminuria is the most interesting of all. This form shows a remarkable daily cycle, the urine being albumin-free at night and when the patient is flat on his back, but appears when he stands up. The terms orthostatic or postural are therefore more suitable. It is the history, extending over considerable time, and the negative physical examination which permit us to place these cases among the functional albuminurias. Other cases of albuminuria may be beautifully cyclic, yet definitely pathological. Such is seen in cases of beginning nephritis, and during the convalescence it is, in fact, a very suggestive sign of Bright's disease.

The rest of the cyclic cases may be subdivided: into those associated with vasomotor phenomena and with the neuropathic element predominating; those with circulatory derangement, as congenital floating kidney; and the hereditary form (Mix). As a rule, the albumin appears after rising, and reaches a maximum at noon or from 4 to 6 P.M., then declines, disappearing from 8 to 10 P.M. If the patient change his habits, the cycle will change as well. Many cases bear little or no relation to meals. There is not only a cycle of the albuminuria, but also diminution in water output, and the sequence each time is, increase in pigments, of albumin, of uric acid, lastly, of urea (Teissier). While casts are rare, yet careful search will, as a rule, show them. The albuminuria may even be diminished by exercise and fatigue, hence is less at night after a hard day's work. Mix[121] has divided such cases into the intermittent and continuous, the terms applying to the periods over which the daily cycle occurs. In the continuous form the cycle continues for years, and if it ceases does not recur. These cases practically never develop in Bright's disease. The adults are neurasthenics with vasomotor paresis, and

[119] Deutsches Arch. f. klin. Med., 1903, Bd. 78, p. 541.
[120] Am. Jour. of the Med. Sci., 1903, vol. cxxvi.
[121] Ibid., 1904, vol. cxxviii. p. 307.

the children in about 37.5 per cent. of the cases have a congenital movable kidney.

There has been a great dispute whether such cases should be considered as pathological or not. They are rare. Other transitory albuminurias are certainly pathological; *e.g.*, those of fevers, and even in these cases is there not always a subsequent history. Most writers warn against considering them as functional, since the duty of renal epithelium is to retain albumin, and when it does not do so something is wrong. Senator says the patients are chiefly young people at puberty of not the best health; they are anæmic, weak, and faint easily. Armstrong,[122] from the study of over three thousand school-boys, found this form in 12 to 15 per cent. It is found more in summer than in winter; heredity is often present; it is often associated with depression of spirits and fainting spells, especially while the boy is standing idle, not when occupied; the boy is apathetic, with a heart subject to intermittent attacks of dilatation and palpitation; it lasts only during puberty. Posner's case was well after seventeen years. Such may, perhaps, be considered as persons renally weak, hence like certain cases of glycosuria, and yet who give no subsequent history. Senator [123] still insists that the majority of these cases are nephritis either at onset or during a latent case. Krehl, having followed several cases over a long period of time, considers that the absence of subsequent history of nephritis allows us to consider the condition harmless; that these are not mild cases of Bright's disease. Broadbent [124] has never known a true case of this form to develop actual renal disease. In all the above cases the amount of albumin is small, the amount of urine normal, with the specific gravity normal; a few hyaline casts are sometimes present; in others no casts are found at any time after careful search; and there are no cardiovascular changes. The immediate cause is much in dispute. Perhaps the circulatory changes due to change in position, which certainly do occur, is the most reasonable. Edel, in three very interesting cases, called attention to the fact that the albumin-free intervals in these cases were also periods of diuresis. This was the afternoon, as a rule, because of the mid-day meal, and the amount of albumin varied roughly inversely to the amount of urine, hence diuresis was the chief thing to strive for. He emphasized the relation between the condition of the urine and that of the heart, the albumin being absent when the pulse was "full." This question is best studied by Erlanger and Hooker,[125] who found that the amount

[122] Brit. Med. Jour., 1904.
[123] Deut. Arch. f. klin. Med., December 8, 1904.
[124] Brit. Med. Jour., 1904.
[125] Johns Hopkins Hosp. Rep., vol. xii., 1904.

of albumin varied inversely as the pulse-pressure (the difference between the maximum and minimum arterial pressure), appearing when this was low.

In other cases, however, originally considered as belonging to this group the continuance of albumin with the presence of casts and the appearance of subsequent cardio-vascular changes marks the case as one of Bright's disease from the onset.

The HYPOSTATIC ALBUMINURIA of splenic origin occurring in some persons with enlarged spleens while recumbent, and absent while erect, is, Rolleston thinks, the opposite of cyclic albuminuria. Since it is not seen in all with enlarged spleens nor in those with the largest, some other factor is necessary. The pressure on the left renal vein may explain it. It may resemble the albuminuria in the chronic passive congestion of mitral disease.

ALBUMINURIA MINIMA (Lecorché and Talamon).—Under this group are included cases with a constant trace of albumin, almost never 0.5 gm. per litre. The output is quite constant in amount, varying little with the position of the patient, the time of day, diet, etc. For each case, however, there may be some factor which causes an increased output. Some such cases are quite certainly the result of a preceding acute nephritis, a residuum as it were. Their prognosis is uncertain and must be guarded, for some develop to true nephritis. Others remain the same for years with no further symptoms.

Under this group the French put the post-infectious cases, albuminurie résiduale, albuminurie paracellaires (or insular nephritis), albuminurie cicatricielle (due to imperfect healing, leaving a "scar"); also the albuminuria of adolescence, the hereditary form, albuminurie phosphaturique, and the albuminurie prégoutteuse.

INTERMITTENT ALBUMINURIAS are those in which periods with albumin are followed by others with clear urine. This term does not include the cyclic or postural, which terms are limited to those with daily periodicity, while the periods of the intermittent may extend over weeks and months or years. The term "intermittent" is more applied to cases of temporary albuminuria due to a known cause. These cases are usually of insidious nephritis, and give a history of some acute infectious disease. But one of the best illustrations is the albuminuria accompanying heart disease, the patients admitted many times with broken compensation and albumin and casts, but soon are albumin-free.

The *intermittent hereditary form* includes, according to some, many cases of the albuminuria of adolescence, the cases showing none in adult life except in response to fairly adequate cause.[126] In some

[126] Dieulafoy, Loude, Arch. gén. de méd., n. s., II, 3, p. 257, 1899.

cases the parents were albuminuric during youth, while of others the neurotic family history alone is evident.

Traumatic Albuminuria.—Transitory albuminuria follows injury to the brain, apoplexy *e.g.;* after injuries crushing the kidneys the albuminuria and casts may continue for a long time with no other signs of nephritis. This may explain some cases of benign latent contracted kidneys (Stern, Curschmann). Menge[127] found that even bimanual palpation of the kidney in physical examination will in 15 of 21 cases cause a transitory albuminuria lasting usually from one to twenty-four hours at the most, and in some cases a slight hæmaturia. Anything obstructing renal venous flow, as in movable kidney during the crises, may cause albuminuria and cylindruria.

Febrile Albuminuria.—During any acute fever, but especially pneumonia, typhoid, malaria, acute articular rheumatism, grippe, or even tonsillitis, there may be a slight albuminuria, simultaneous with the rise in temperature and disappearing with its drop. In such cases the cloudy renal epithelium, the faintest grade of inflammation (Leyden), is considered the anatomical basis. The amount is usually small, but sometimes much. Hyaline and epithelial casts are sometimes found, but no other formed elements indicating inflammation. In general it is only a matter of degree which separates such from true cases of nephritis.

Under **hæmatogenous albuminuria** is included a very confusing group of non-febrile cases without at autopsy renal lesions, except, perhaps, slight parenchymatous changes. Among this group are purpura, scurvy, chronic lead or mercury poisoning, lues, leukæmia, cachexias, and anæmias in which the albuminuria is always slight and occurs only when these conditions are severe, cholæmia, glycosæmia, and after ether and chloroform narcosis.

Strictly speaking, "hæmatogenous albuminuria" should mean one in which, either due to some alteration of a normal proteid of the blood, or because it is foreign, a proteid unsuitable for use is excreted. All cases with the possibility of the presence of a toxic influence on the kidneys, for instance, lead, mercury poisoning, etc., should be excluded, since such would have a truly renal origin. It is true that foreign proteids in the serum are excreted, *e.g.*, albumoses, egg albumin, peptone, casein, free hæmoglobin, etc. Some consider that in all cases of albuminuria such is the case, an abnormal proteid or a normal one rendered unfit for further use being merely excreted. Yet in cases of true nephritis there is no evidence of a foreign proteid or qualitative change of the normal proteid. It is suggested, however, that quantitative changes either of proteids or salts could explain the albuminuria. But more probably the real cause is in the cells them-

[127] Münch. med. Wochenschr., June 5, 1900.

selves, the renal epithelium being exceedingly sensitive to changes in its nutrition, and that a true hæmatogenous albuminuria is not proved.

The Nervous Form.—Epilepsy, apoplexy, tetanus, exophthalmic goitre, injuries to the head, delirium tremens, various psychoses, even neurasthenia and migraine may be accompanied by a slight transitory albuminuria. In some cases there are a few casts present. Interestingly enough in other cases only casts are to be found. We followed the urine of such a case in a boy fourteen years old with hysterical attacks. A very transitory albuminuria cannot be excluded, since the urine may not have been examined early enough. The cylindruria lasted for several weeks.

Cases of closure of the ureter, retention of urine in the bladder, compression of the thorax, have been accompanied by albuminuria; digestive disturbances, as obstruction of the bowel (a reflex cause being assumed as in cases of strangulation of bowel or omentum;[128]) acute diarrhœa, constipation, and liver disease are sometimes given as causes. In two-thirds of the cases the albumin disappears after the obstruction is relieved even though the bowel has been rendered gangrenous. The cause is uncertain. It is probably not the absorption of any bodies, since in peritonitis, where there would be a similar absorption, the albuminuria does not occur. Such cases are transitory.

Albuminuria with Definite Renal Lesions.—In active renal congestion, as after exposure to cold, or in chronic passive congestion due to heart or lung disease, tumors, or pregnancy, albumin may be present, yet no other renal lesion found. As a rule the albumin is little and its amount is parallel to the amount of urine, while in the case of true nephritis the amount varies inversely as the amount of urine as a rule. In children albuminuria may accompany the simple hyperæmia in diphtheria, *e.g.*, which may then stop or develop into a nephritis.

Organic Bright's disease of all varieties is accompanied by albuminuria at some time during its course. It is interesting that there is no parallelism between the amounts of albumin and the severity of the nephritis. In the chronic interstitial nephritis ending in uræmia it may be present in traces. In other cases periods with traces may alternate with months when there are none. In general the rule is that the more acute the case the larger the percentage of albumin. In all cases it is, however, more a matter of percentage than of total albumin; for to excrete a larger amount of urine with a lower percentage of albumin is evidence of a better renal condition than previously when the percentage was higher but the total output much smaller since the output of urine was diminished. In some cases definitely acute there may be no albuminuria.[129] In nephritis also the

[128] Neumann, Trans. Clin. Soc. of Lond., 1897, Bd. 30, p. 65.
[129] Herringham, Trans. Clin. Soc. of London, vol. xxxiv. p. 901.

percentage of albumin varies, as a rule, inversely to the amount of urine. The albumin is seldom present in amounts of more than 1 per cent. Sometimes it reaches 2 per cent., while in very rare cases 5 per cent. or, in one case, 8 per cent. Senator mentions a case of subacute nephritis with a percentage of from 6 to 8 per cent. over a period of some days. Cases with the largest amounts of albumin output are interesting enough often due to lues. These cases of nephritis syphilitica acuta præcox are rare, but between 20 and 25 are recorded. In Hoffmann's case the enormous albuminuria ran parallel to the luetic symptom and improved under mercurial treatment.

Salkowski's case [130] is especially interesting. The urine had a specific gravity of 1056, and 7 per cent. proteid. On standing, there was deposited a rich white amorphous precipitate, not a coagulum, of a proteid giving reactions between globulin and an albuminate, and which after standing gave those of albumin. This same case on another day showed even 8.5 per cent. albumin (the blood contains but about 7.5 per cent. of proteid).

The total output of albumin is seldom great, that is, more than from 1 to 20 gms. The deleterious effects of the nephritis cannot be attributed to the actual loss of albumin, since this loss, as a rule, can be easily covered by one good meal. In amyloid disease the albumin may be much or almost none; as a rule from 0.5 to 0.05 per cent.

This albuminuria varies much, there being definite waves in the output. At first indiscretion in diet increases it, probably by intensifying the acute element of the process, later a more liberal diet may improve the condition. In some cases it would seem as if meat were not as harmful as vegetables, perhaps due to the salts of the latter. The albumin is increased by the erect posture, but this does not explain its increase during the waking hours, since the same curve is presented by patients who are semierect all the time as by those who can rest recumbent. Exercise of any kind, even massage (Edgren), increases the output.

A pure milk diet sufficient to cover the heat-needs is injurious, causing even hæmaturia, and should be varied with other nitrogenous foods.

"Hetero-albumosuria." Bence-Jones' Body. "Kahler's Disease." "Myelopathic Albuminosuria of Bradshaw."—This body, which occurs in certain rare conditions in the urine in very large amounts, was supposed, from some of its chemical properties, to belong to the hetero-albumoses. Some recent work, for instance that of Magnus-Levy who obtained it crystalline, showed that it is nearly related to genuine albumin. Among other reasons for this is that its digestion products include all the primary proteoses except hetero-

[130] Berl. klin. Wochenschr., March 3, 1902.

albumose. This throws considerable doubt on the belief that this is a primary digestive product of digestion. Lindemann concludes that while it cannot without objections be put in any group of proteids it is nearest the true albumins. Dechaume considers it a mixture of at least three proteids (or groups of proteids),—proto-albumose, dysalbumose, and a body like hetero-albumose. In 1903 but about 35 well reported cases had been published. In all but one (Askanasy), and this a case of lymphatic leukæmia, the condition has been of multiple myelomata. In all cases there is extensive disease of the marrow. Such cases run a rather acute course with a fatal termination in from one-fourth to one and a half years.

The Bence-Jones' body is present in large amounts often, even 7 per cent., but the majority below 1 per cent. Some cases are reported as intermittent (Boston). Coriat [131] reported a case with none in the urine, but 4 per cent. in the pleural fluid.

Reactions.—The specific reaction is that on warming there develops at a low temperature (about 60° C., often 52°) a milky, then heavy, sticky precipitate, which disappears for the most part and in some cases perfectly on bringing to a boil, and reappears on cooling. The urine must first be rendered acid with acetic acid. This being the characteristic reaction, the name suggested by Hugounenq for the condition "thermolytic albuminuria" ("albuminurie thermolytique") is very appropriate.

On adding nitric acid to the urine a heavy precipitate forms which is soluble on warming and reappears on cooling.

The urine may be saturated with $(NH_4)_2SO_4$ at 100° C., filtered; the precipitate washed with saturated $(NH_4)_2SO_4$. The precipitate is then dissolved in water or dilute NaCl solution and the biuret test applied. The urine gives the biuret reaction directly.

These are most striking reactions and attract attention at once. The precipitate appears at a moderately low temperature, which depends on the amount present, also on the salt content of the urine. While a definite temperature cannot be stated, it is in general below 60° C. In the different cases the properties of the substance found have differed so much that either they were different bodies or were not tested pure, or, what is the present view, the various amounts of salts and urea affected the tests. The urine may be turbid when voided.

Boston [132] proposed the following test based on the large amount of loosely bound sulphur it contains. From 15 to 20 cc. of urine in a test-tube are mixed with an equal amount of saturated NaCl and shaken to a perfect mixture. Then 2 to 3 cc. of 30 per cent. NaOH are added and the tube shaken hard. The urine at the top of the tube

[131] Am. Jour. Med. Sci., 1903, vol. cxxvi.
[132] Ibid., 1902, vol. cxxiv.

is then heated to boiling and PbAc solution (10 per cent.) added drop by drop, heating after each drop. In one-half to one minute one gets a brown color turning to black.

The output of this body is quite constant during the day and is not affected by diet, hence it is probably not a non-assimilated product of digestion. It seems to be formed in the bone-marrow. Some connection with the granules of the myelocytes and tumor cells is suggested.

This albumose can be demonstrated in ascitic fluid, blood, and bone-marrow.

Quantitatively the Esbach tube will give an approximate determination.

For recent literature concerning the nature of the substance the reader is referred to Simon.[133]

Albumosuria, "Peptonuria."—Under this term at least two different groups of bodies have been described,—the above-mentioned rare so-called "Bence-Jones' body," which because of its many reactions was counted with the primary proteoses, and a group of bodies having nothing in common with the above, formerly called peptones, a name based on Brücke's definition as a proteid not precipitated by K_4FeCN_6 and acetic acid. By "peptone" is now generally understood one not precipitated by complete saturation with $(NH_4)_2SO_4$ (Kühne), and judged by this standard these bodies are chiefly deutero-albumoses, hence the name "peptonuria" is less used and "albumosuria" has taken its place. Yet this criterion is not satisfactory (Neumeister); also true peptone judged by it has been demonstrated in the albumosuria of croupous pneumonia and other diseases, as the puerperium, ulcer of the stomach, and pulmonary tuberculosis. It occurs always with albumose (the reverse is not true).[134]

In testing for the deutero-albumoses the urine should be albumin-free, and if this is not the case it should be made so by the Hofmeister method. Mörner's body may be precipitated by basic lead acetate.

An easy test is to saturate the urine with ammonium sulphate, a flocculent precipitate indicating albumose.

A good preliminary test for the deutero-albumoses is that of Hofmeister. To the urine is added one-fifth volume of concentrated acetic acid and then phosphotungstic acid. If the urine remains clear after standing for some time, these bodies are not present, while a milky cloud at once or in about ten minutes indicates them. This test is valuable if positive, but not if negative.

The biuret test is that usually used. According to Hofmeister the albumose is first precipitated with tannic acid or phosphotungstic

[133] Am. Jour. Med. Sci., 1902, vol. cxxiii. p. 939.
[134] Ito, Deutsches Arch. f. klin. Med., 1901, vol. lxxi.

acid. The precipitate is dissolved in a little water and the concentrated solution tested. NaOH or KOH are added in excess and then very dilute $CuSO_4$. Deutero-albumoses are indicated by a violet-red color. It may be necessary to filter off the precipitate of $Cu(OH)_2$. If this test be applied to the urine directly, the color obtained is a red or a reddish-brown, the violet being obscured by the color of the urine. The test may also be performed as a contact test, the urine being rendered alkaline and then carefully superimposed by a dilute $CuSO_4$ (5 cc. of a saturated solution to 1 litre of water).

For a positive test the albumose must be isolated. This may be done with phosphotungstic acid, which will allow 0.1 gm. per litre to be detected, or tannic acid, somewhat less delicate. Albumin and "nucleo-albumin" must first be removed with basic lead acetate.

Salkowski's method. This was designed to detect small amounts of albumoses. To 50 cc. of urine in a beaker is added 5 cc. of concentrated HCl or acetic acid. It is then precipitated with phosphotungstic acid and warmed over the free flame; the precipitate collects as a tough resinous mass at the base of the beaker. The supernatant fluid is decanted and the precipitate washed with distilled water a few times (twice), being careful that none be lost. On the precipitate is then poured 8 cc. of water plus 0.5 cc. of NaOH (sp. gr. 1.16). It dissolves readily. The blue solution is then warmed until clear. More NaOH is added if necessary, since the mixture is often of a dirty grayish-yellow color and cloudy. The solution is then cooled and the biuret test applied by adding in a test-tube a few drops of 2 per cent. $CuSO_4$. Before applying the biuret test Sahli recommends that the colored fluid be cleared with $BaCl_2$. Urobilin, if found with the spectroscope, must be removed, and may be extracted with amyl alcohol. Sahli says that it is completely enough precipitated with $CaCl_2$. The test may be performed in five minutes. The small amount of urine used minimizes the danger of mistake with "nucleo-albumin."

Another method is as follows: To the urine is added 0.1 volume of concentrated HCl, then PWo acid, again HCl, and again PWo acid, until neither gives any more precipitate. The urine is then filtered at once before uric acid precipitates. The precipitate is washed on the filter with H_2SO_4 (3 to 5 volumes concentrated H_2SO_4 in 100 cc. of water) until the wash-water runs colorless. The moist precipitate is then rubbed up with dry $Ba(OH)_2$ in excess. A little water is then added and the warm solution filtered. If heated too much the solution becomes dark. The peptone solution is always yellow. The biuret test is then applied and a red color obtained if albumose be present. In this case the test is best performed as a contact test, since the $BaSO_4$ precipitates and settles.

Hammarsten recommends the following method, which has been modified by Bang: Ten parts of urine plus 8 parts of saturated ammonium sulphate are heated to boiling for a few seconds. The hot fluid is then centrifugalized from one-half to one minute and decanted. From the precipitate is extracted the urobilin with alcohol. The residue is then taken up with little water, heated to boiling and filtered. This removes the albumin. It is then shaken out with chloroform to remove the last trace of urobilin. The chloroform is then pipetted off and the water tested with the biuret for the presence of albumose. This is a very practical clinical method.

Alder,[135] after criticising all the preceding, recommends the following as more accurate. Albumin if present is removed by trichloracetic acid (15 per cent.). To 6 to 10 cc. of urine in a test-tube are added 1 to 2 drops of HCl till acid, then 5 per cent. phosphotungstic acid till complete precipitation. The fluid is then centrifugalized for a few seconds. The supernatant fluid is poured off, the sedi-

[135] Berl. klin. Wochenschr., 1899, pp. 764, 780.

ment suspended in absolute alcohol, and again centrifugalized. This is repeated till the sediment and the alcohol (colored yellow with urobilin) are white and clear. The sediment is then suspended in water, strong NaOH added, shaken till all blue color disappears, then the $CuSO_4$. By this method 0.2 gm. per litre can be detected.

Occurrence.—The deutero-albumoses may occur either alone or with albumin. In cases of nephritis the albumose is said to accompany the albumin, which it may precede or continue after the albumin has disappeared. The reason for this is not known. Since the urine contains a pepsin-like ferment the formation of albumose by the digestion of albumin may be suspected.

The hæmatogenous group may cover all, since when there is present in the blood considerable albumose some of it is excreted; none if the amount is small. The source may be the breaking down of the cells of the blood, as in leukæmia; or of tissue proteid of the body, as during the puerperium, in which case the involution of the uterus is supposed to be the cause, which, however, cannot always be true, since it may occur before delivery; in pregnancy with a dead fœtus; also in some cases with living fœtus, in which case it is said to arise in the amniotic fluid; in cases of hemorrhage underneath the skin, scurvy, purpura, ecchymoses, destruction of red blood-cells or tissue-cells due to toxines.

Enterogenous albumosuria is seen in cases of gastric or intestinal ulcer, as, *e.g.*, in intestinal tuberculosis. In such cases small amounts of albumose ingested, *e.g.*, somatose, will give a positive test; normally larger amounts are necessary (alimentary albumosuria). Some consider that if following the ingestion of from 40 to 60 gms. of albumose this body be found in the urine it is in favor of a gastric or intestinal ulcer.

In nephritis, especially luetic, albumosuria occurs. The "hepatogenous" form occurs in acute yellow atrophy, cirrhosis, cancer, catarrhal jaundice, phosphorus poisoning. The "febrile," in most fevers, especially the infectious; rheumatism, septicæmia, typhoid, phthisis, gangrenous processes, measles, scarlet fever, erysipelas, smallpox, especially as the temperature falls. Mental diseases, especially paralyses. "Pyogenic albumosuria" is supposed to be due to the absorption of an exudate, as in pneumonia during resolution; empyema, bronchiectasis, epidemic cerebrospinal meningitis, abscess, and osteomyelitis. Gangrenous processes everywhere cause it, also cancers of any organ, from increased destruction of tissue and tissue poisons (histogenic form).

The common element in most of these conditions is the breaking down of some tissue or exudate, *i.e.*, increased catabolism such as occurs in all fevers and in cancers (Aldor) or exudates.

There are certain sources of albumose which should always be excluded; as, for instance, spermin and secretions of the accessory

genital glands; the foods, since on a milk diet in nephritis it is claimed that the products of digestion are absorbed and excreted unchanged; and lastly, that due to technique in removing albumin from the urine. Clinically, the deutero-albumoses are important only when the urine is albumin-free. In albuminuria it may nearly always be demonstrated and the question arises whether it was preformed or formed from the albumin by the technique. The amount formed in this way, however, if the work be done well should be very small.

It has very little clinical value, since it has such a wide occurrence. It could be of value, however, in a case of suspected abscess (*e.g.*, of the appendix, brain, or an empyema); or in the differential diagnosis of tuberculosis and epidemic cerebrospinal meningitis. The amount is always small when compared with that of the Bence-Jones' body.

Hæmaturia.—This may be a symptom of the following conditions:

(1) General diseases: the malignant forms of acute specific fevers, especially smallpox, typhoid fever, malaria; in leukæmia occasionally; in the so-called hemorrhagic diathesis, hæmophilia, scurvy, morbus maculosus Werlhofii, and the purpuras. In the latter diseases the process may be limited to the kidney.

(2) Renal causes, acute and chronic congestions, and inflammations of the kidney; all nephritis cases at the onset, there being one form called the "hemorrhagic" form. Those due to turpentine, carbolic acid, and cantharides especially have an hemorrhagic onset. In purulent nephritis traces only of blood may be present. The chronic parenchymatous, Weigert considered always hemorrhagic, hence small amounts of blood in the urine may always be expected. In amyloid disease there are few or no red blood-cells. In chronic passive congestion due to different causes there may be blood in the urine. In renal infarctions it may be considerable, but that is rare; in new growths of the kidney sometimes the hæmaturia is profuse; at the onset of tuberculosis, especially when the papillæ are involved; in cystic kidneys, renal calculus, and, lastly, parasitic diseases of the kidney, especially filaria, echinococcus and the distoma hæmatobium. In congestion due to venous thrombosis, *e.g.*, of the new-born, hæmaturia is said to be a common symptom.

(3) It is also found as the result of lesions or diseases of the urinary passages, as stone in the ureter, tumors and ulcers of the bladder, parasites of the bladder, calculi and ruptured veins; in urethritis.

(4) In trauma of any part of the urinary tract from the kidney down.

(5) And lastly an interesting group with no known lesion; the so-called "Gull's renal epistaxis" or "essential renal hæmaturia," or

"angioneurotic hæmaturia," or "renal hæmophilic," is a rare disease of middle adult life, often unilateral. In certain of these cases angiomata of the kidney have been found, in others none, and nervous causes are suspected. Some of these cases recover without further treatment; others after treatment of the nervous system, while others after a nephrotomy, or nephropexy or simple exposure of the kidney.[136]

In women the vagina as a source must always be excluded.

More recent work with microscopic examination throws some doubt on the normal nature of these kidneys. Eshner[137] collected 48 cases of unilateral renal hæmaturia, most of which had been diagnosed as calculus or cancer. Since then other interesting cases have been reported. A diagnosis of unilateral hemorrhagic nephritis was made in Stich's case.[138] In Schüller's case the kidney looked normal, but microscopically chronic parenchymatous nephritis was found.[139]

The term "hæmaturia" is used only when blood is grossly visible. The urine is always turbid, of a light smoky to a bright red or blackish-brown color. Microscopically are found the red blood-cells in various conditions of preservation, and other elements according to the cause. In renal hæmaturia clots are seldom present, the urine and blood are homogeneously mixed hence in equal amounts in the two-glass test, while in cases of hemorrhage from the bladder the second glass will contain the more blood, and if the bladder be washed out the washings will be blood-stained, while in renal cases, clear. In acute exacerbations of a chronic parenchymatous nephritis especially, the amount of blood in the urine may be considerable. Clots are present in rare cases, as when large vessels of the kidney rupture, or in cases of aneurisms, trauma, or varices. In a case in the ward recently a clot four inches long, a cast of the ureter, was voided. Such clots are more common in cancer than in calculus.

Gerhardt thinks that the blood-cells from the kidney are more spherical, more leathery in color than usual, while all the morphological elements from the kidney, the casts and epithelial cells, are yellowish-brown. In renal hæmaturia are found also casts of various kinds, blood-casts, or casts with red cells attached, and renal epithelium, showing parenchymatous lesions. Albumin will also be present. It is generally believed that if the blood be not from the cortex and the urine allowed to settle, the clear supernatant fluid will be albumin-free.

Hæmoglobinuria is the result of hæmoglobinæmia, or the destruc-

[136] Stavely, Johns Hopkins Hosp. Bull., March, 1893.
[137] Am. Jour. Med. Sci., 1903, vol. cxxv.
[138] Mitth. aus d. Grenzgeb. d. Med. et Chir., 1904, Bd. 13, p. 781.
[139] Wien. klin. Wochenschr., 1904, No. 17.

tion of red blood-cells within the blood-stream in such numbers that the body cannot warehouse the pigment, which is therefore excreted by the kidneys. This occurs when about one-sixtieth of the total hæmoglobin is set free. This may follow various blood poisons, potassium chlorate, pyrogallic acid, CO, naphthol, AsH_3, etc. Or the poisons of fevers,—scarlet fever, typhoid, yellow fever, especially malaria, lues; severe burns, exposure to cold, transfusion of foreign serum; also during pregnancy (Brauer); as an epidemic fever of the new-born, in certain cases of nephritis, and after severe intra-abdominal hemorrhages.

Curry[140] emphasizes the point that in "black water fever," so commonly supposed to be of malarial origin, evidence of malaria is not always present before or after death. The black water fever due to malaria may later recur after an ordinary dose of quinine.[141] The urine always contains albumin, and the albuminuria may precede or follow the hæmoglobinuria. (This proteid is said to arise from the red blood-cells.) This preceding albuminuria is a good answer to the theory that it is the irritation by the hæmoglobin which causes this condition. Again, this idea of the origin from a hæmoglobinæmia, although probable, does not rest on a very firm basis, for, as Senator says, hæmoglobinæmia has never been proved in the hæmoglobinuria due to infectious diseases or hemorrhagic nephritis, while in two cases of hæmoglobinuria it was surely absent.

The PAROXYSMAL HÆMOGLOBINURIA is a condition which has attracted considerable attention. This rare condition of adults occurs especially after exposure to cold or exertion, and consists of a hæmoglobinuria often preceded by fever and chills and pain in the lumbar regions. The output of hæmoglobin continues for one or two days or less. The excretion is usually preceded by a hæmoglobinæmia, but in rare cases this has been missed.

The cause for this has been in much dispute. Some claim a hæmolytic action of blood-plasma, "an increased number of complements," others a chemical toxine, others a mechanical injury of the red blood-cells, and in the circulation shadows are found, while others think the cause is in the kidney. Senator thinks this latter is to be considered in many cases. It is of interest that 23 of 77 cases gave a history of lues. The urine is red or dark brown; spectroscopically, is found methæmoglobin alone or with hæmoglobin; microscopically, are found amorphous blood pigment in masses or casts, or even crystals of hæmatoidin; few or no red blood-cells will be found, and if present they are so few that they cannot explain the pigment; often hyaline and granular casts and renal epithelium are present; sometimes many calcuim oxalate crystals also; albumin is always present, and often bile pigment, but, it is said, no bile acid. As the hæmoglobin disappears the albuminuria will continue for a short time. During the attack will be found in the blood often shadows of red blood-cells, increased leucocytes, amorphous masses of pigment, and a great many platelets. Sometimes the hæmoglobinæmia

[140] Jour. Am. Med. Assoc., May 3, 1902.
[141] Nansen, Brit. Med. Jour., May 16, 1903.

can be seen even grossly, the plasma having a reddish or a ruby-red color. It is doubtful if the isotonicity of the blood is changed. Degenerations in the red blood-cells are common, and other points indicating their lowered resistance, for instance their resistance against shaking and against CO_2. Donath[142] was unable to show any lowered resistance of the cells to any mechanical influence.

The immediate causes are various; among them are excessive exercise or mental excitement. Cold is the most potent, and the patient may produce it by plunging his hands into cold water. It may also be produced locally by tying a string about one finger. Homburg's patient[143] showed it after an involuntary cold plunge of three minutes' duration.

In hæmoglobinuria the urine may be clear, but it is usually more or less clouded by hæmoglobin casts, amorphous masses of pigment, and casts from the associated nephritis. If it be sedimented, the supernatant urine is a clear blood-colored fluid, and in the sediment so few red blood-cells that they could not possibly explain the amount of hæmoglobin. The urine must be tested fresh to determine the difference between these two conditions, since the red blood-cells will so quickly go to pieces, freeing much hæmoglobin and leaving an abundant grayish-brown albumin-rich sediment, in which may be seen the stromata of the laked red cells. Stempel reviews the literature to date in a splendid résumé.[144]

Chemical Tests.—These, apart from the spectroscopic, are the same for hæmoglobin and its many modifications, and whether intracellular or not. This last point can be tested only by microscopic examination.

(1) An ordinary *heat-acetic acid* albumin test is made. A brown coagulum forms which usually swims on the surface. If this be shaken with acid alcohol (H_2SO_4), the clot is decolorized. The color depends on the amount of hæmoglobin present. This test is not very delicate.

(2) *Heller's Test.*—A test-tube is filled half-full of urine, about five drops of NaOH added to make strongly alkaline, and then warmed to form hæmatin. A brownish red or bloody precipitate results of the precipitated phosphates and carbonates of the alkaline earths which carry down the hæmatin. If the urine be already alkaline, the phosphates of the alkaline earths may already have precipitated, and hence the test fail, in which case it is necessary to add a certain amount of normal urine in order to supply these salts.

This test is very delicate, indicating 1 cc. of blood in 1 litre of urine. If the fine red blood-color of the precipitate cannot be seen, since the urine is dark or jaundiced, it should be filtered off, the precipitate dissolved in acetic acid; a red solution is obtained which decolorizes gradually in the air. This red precipitate is by reflected light of a greenish tinge. If but little hæmatin be present, the pre-

[142] Zeitschr. f. klin. Med., 1904.
[143] Ibid., vol. liii.
[144] Zentralbl. f. d. Grenzgeb. d. Med. et Chir., 1902, vol. v. pp. 177, 267.

cipitate should be dissolved in acetic acid and the residue of this used for the Teichmann's test. Similar red precipitates may be obtained after the ingestion of senna, rhubarb, or rhamnus. The urine, however, is yellow at first, and on the addition of the sodium hydrate becomes red. The phosphate precipitate, if dissolved in acetic acid, gives a lemon-yellow solution, which changes on exposure to the air to a violet. Hæmatoporphyrin and other pathological pigments may give a red precipitate, but the spectroscope will quickly indicate the difference by showing the alkaline hæmatin spectrum. If but a trace of blood be present the urine is first made alkaline with NH_4OH and then precipitated with tannic acid. The precipitate is used for the hæmin crystal test.

(3) *Teichmann's HCl-Hæmin Test.*—The precipitate obtained by either of the preceding tests, or, better, a tannic acid precipitate, is filtered, washed, and dried in the air. A very small granule of the dry precipitate is put on a slide with one granule of NaCl and a few drops of glacial acetic acid. The cover-glass is then put on. The specimen is then warmed over a small flame so that the acetic acid steams. The acid is constantly renewed. When the acetic acid sur-

FIG 31.—Hæmin crystals. × 400.

rounding the granule is stained a brownish color, the heating is discontinued and the slide cooled very slowly. The characteristic crystals of hæmatin may soon be seen with the microscope.

This test requires care. The reasons it so often fails are that the specimen is heated too hot (a simple steaming is sufficient), or that the acetic acid is not sufficiently renewed, or the slide is cooled so rapidly that it forbids good crystallization. Good crystals may be obtained if the specimen is not heated at all but allowed to stand for twenty-four hours.

(4) The tannic acid precipitate mentioned in test (2) may be ashed on a platinum-foil, the ash dissolved in a few drops of hot HCl, this diluted and filtered and tested with the potassium ferrocyanide solution.

(5) The *Guaiac test* (Schönbein-Almén test) is very delicate. The urine is overlaid carefully with a mixture of equal parts of Guaiac tincture (alcoholic solution of resina Guaiaci, 1 to 5) and old oxygenated oil of turpentine. The turpentine should be exposed to the air for some

time, that it may be well oxygenated; the Guaiac tincture should not be exposed to the sun or air, and should be kept in a colored bottle. These solutions when mixed should show no blue color. The urine is superimposed with this, and if blood be present an intense blue ring appears at the line of separation.

The urine must be acidified with acetic acid if necessary. This test is so delicate that it may be positive when the spectroscopic test is negative. Pus need not be excluded unless the above solutions have not been properly kept. The test should always be controlled with a fluid known to contain blood. The test is not absolutely positive, since other bodies will give it, yet it always has a negative value, since if negative no blood is present.

Spectroscopic.—The student should be well trained in the use of the spectroscope. The blood spectra which are important are those of oxyhæmoglobin, reduced hæmoglobin, and methæmoglobin. In the case of the urine a mixed spectrum may usually be expected. If the blood is fresh, that of oxyhæmoglobin will predominate, but in hæmoglobinuria, or in nephritis, methæmoglobin. Bacteria will oxidize the last two to oxyhæmoglobin. The urine should be diluted if necessary, and must be clear.

Very small amounts of blood-pigment are detected as follows (Hoppe-Seyler): To 100 cc. of urine is added an albumin solution or an albuminous urine. This is heated, that a good coagulum may form, the precipitate washed, pressed out, and rubbed up with alcohol which contains a little H_2SO_4. This is then warmed and filtered. The filtrate after treating with NaOH and $(NH_4)_2S$ gives the bands of hæmatin.

Methæmoglobin.—Many previous observations of this are not reliable, since only a single spectroscopic examination was made, and hæmatin may have been mistaken for it. It is present in all fresh urines containing blood, although later it may be oxidized to oxyhæmoglobin. For its detection the spectroscope is necessary, but not alone the spectrum of neutral methæmoglobin, which may be confused with hæmatin, but ammonia must be added and the spectrum of alkaline methæmoglobin obtained. This spectrum may be confused by other bodies which give bands or which darken the field as bile or urobilin. One must be careful not to dilute too much, since it is easy to pass the point at which the lines are seen.

Urobilin or bile-pigment may be removed with basic PbAc, the hæmoglobin remaining in solution, but methæmoglobin will be precipitated. In case red blood-cells are present, water should be added in sufficient amount to lake them. If no absorption bands are seen, or they are very faint, the hæmoglobin may be transformed to reduced hæmatin, whose spectrum it is easier to study. This reduction is best done with $(NH_4)_2S$, or with $NaHSO_3$ and zinc, for only a short

time, else the albumin will be precipitated. The spectrum of reduced hæmoglobin is fainter than that of oxyhæmoglobin. If a few drops of concentrated NaOH be added, hæmatin is produced.

Hæmatoporphyrin.—Hæmatoporphyrin, an iron-free derivative of hæmoglobin, is present in the normal urine in very slight traces, but sometimes in very large amounts. Sulphonal is one of the most important causes of the excretion of large amounts of this body. It is present in larger amounts, however, in cases of rheumatism, pericarditis, Addison's disease, paroxysmal hæmoglobinuria, cirrhosis of the liver, pneumonia, and hæmatemesis. Some have considered that for the diagnosis of liver disease the increase is important. It is increased in lead poisoning. It is also increased in acute infectious diseases and in various forms of tuberculosis. Stockton thinks it was due in his case to acute inflammatory processes of the cord and spinal nerves.

The color of the urine is sometimes deceptive. This is especially true of the cases of lead poisoning. In other cases it is dark brownish-red, cherry-red, or Bordeaux-red, varying with the amount of this pigment. "Port-wine" color is a common term.

Following the use of sulphonal about 40 fatal cases have been reported, chiefly in women. Trional and tetronal also cause it.

The cause is rather doubtful, direct action upon the blood being claimed, also hemorrhages into the stomach wall caused by these drugs, and transformed by the gastric juice, the hæmatoporphyrin being reabsorbed and excreted. This is supposed to be true particularly of the lead cases, but certainly is not of all. In other cases the one trouble is said to be a lesion of the renal epithelium. In hæmoglobinuria there is a preceding hæmoglobinæmia, as a rule, but a hæmatoporphyrinæmia has not yet been proved.[145]

Pal [146] reports a case of paroxysmal hæmatoporphyrinuria with "black" urine, with symptoms similar to those of paroxysmal hæmoglobinuria, and which he thinks may be due to lues.

Garrod [147] collected 12 cases not due to sulphonal. In this group of cases the most were of males, and the condition lasted even years without bad symptoms. For these cases he mentions as generally held the opinion that the condition is due to perverted catabolism of hæmoglobin rather than increased destruction of red cells.

SEDIMENTS

Preservation of the Urine.—To study the sediments, especially the organized, it is best that the urine be examined while perfectly fresh. Casts will disappear to a certain extent in any urine, especially if the patient receive the organic salts as diuretics. We often see the urine alkaline and all casts gone one hour after voiding. The urine is best

[145] Ruedy, Am. Jour. Insanity, October, 1899.
[146] Centralbl. f. inn. Med., 1903, vol. xxiv. p. 601.
[147] Lancet, March 5, 1904.

centrifugalized. In case the immediate examination is impossible, or a centrifuge is not at hand, the urine may stand in a conical glass, the cavity of which ends at a sharp point, or may be filtered through a filter paper and the last drops examined. A drop of the sediment is drawn up into a pipette; the outside of this is then wiped off and a drop blown onto a glass slide. In case there is very much sediment in the glass, the components of the different layers will vary considerably, since their specific gravity varies so, hence the sediment must be first stirred up or several specimens examined.

To preserve the urine from bacterial action during long sedimentation, a piece of camphor may be used, or one-fifth volume of 1 to 200 chloroform water, or one-fifth volume of saturated borax solution, which prevents the precipitation of the urates and preserves the cells, while it does not coagulate the albumin. A few drops of formalin are really best, but this may add to the sediment a component of its own. One cc. of pure chloroform, so valuable in the preservation of urine for chemical work, is not to be recommended. Thymol is fairly good. To use a clean pipette is very important, so many times are mistakes made by using one in which elements of a previous sediment remain. In case the bacteria are the subject of study it is well to add two volumes of alcohol, which by lowering the specific gravity hastens the sedimentation.

To preserve sediments for a long time the urine is centrifugalized and the supernatant fluid poured off. To the sediment may then be added chloroform water to preserve crystals, or formalin to 1 to 2 per cent. to preserve casts and formed elements. They keep only fairly well. Of the crystals, the phosphates, carbonates, and ammonium urates keep well; calcium oxalate poorly, uric acid never well.

The student should be early taught that there are very few sediments which may be recognized beyond doubt from their appearance alone, hence the value of microchemical tests with acids, alkalies, Lugol's, stains, etc., which may be drawn under the cover by applying a piece of filter paper to the edge opposite to the drop of reagent. If few casts are present the surface of a large slide is covered by the urine and no cover-glass used.

There is one peculiarity of crystalline sediments worthy of mention,—that the crystals tend to belong to one system; that is, the crystals of one salt are usually similar; they are nearly all of the same regular or irregular form; all the uric acid crystals may be hexagons, all of calcium oxalate ovals, all the triple phosphate flat plates, etc.

Another peculiarity is the relative infrequency of crystalline sediments in women's urine.

The sediments have been divided into the organized and unorganized. By the former are meant tissue constituents, casts, bacteria, and formed elements from the urinary or communicating organs.

The reaction of the urine may often be detected from the gross sediment; when acid this is granular, when alkaline, mucoid. Specimens should be examined when made, since if allowed to dry even a little the crystals which separate are quite confusing.

Unorganized Sediments. (1) **Urates and Uric Acid.**—A precipitation of urates occurs in any concentrated acid urine, especially on a cold day, much to the distress of mind of some persons. The urine first presents a very milky appearance, and the sediment then settles on the bottom and sides of the glass, forming a heavy voluminous mass. The color of the precipitate will vary from a yellow to a bright rose-red. It is soluble in acids with the subsequent precipitation of uric acid, and in alkalies. It is easily soluble on warming. While common in any concentrated scanty normal urine, it is especially so in certain fevers, chronic passive congestion, pneumonia, and rheumatism, seldom in nephritis or in albuminous urines.

One of the most remarkable crystal forms are the long branching rods like huge yellow bacteria, which disappear on warming.

This precipitate is said to be the *quadriurates* (Roberts),—$MH\overline{U}\overline{U}$, which are formed by the action of MH_2PO_4 on the biurates, $MH\overline{U}$; if in sufficient concentration they are precipitated. The quadriurates in solution are easily decomposed to biurate and uric acid, the latter precipitating as little bright red so-called *red pepper granules* on the sides of the glass. This will be the only sediment in case the quadriurate is not in sufficient concentration itself to precipitate. In the urate sediment are found also calcium oxalate crystals, and, as ammonia is soon formed, a certain amount of the acid urates will be dissolved, some will be transformed to ammonium urate, hence in the same sediment may occur ammonium urate, the so-called quadriurates, uric acid and calcium oxalate, and even a few triple phosphate crystals. This transformation occurs progressively from above downward.

This explanation of Roberts is so satisfactory that it is unfortunate that it has little evidence behind it. One thing is quite certain, that the precipitation of the urates is the result of a chemical transformation of the salt, since it precipitates in the cooling urine too slowly to be due to this alone, and warming the urine to the previous temperature does not redissolve it. Also during its formation the acidity of the urine is said to increase.

Microscopically, the acid urate sediment consists of very fine granules in clusters of a yellow to a reddish-brown color which disappear on warming. On the addition of a little acetic acid the subsequent crystallization of uric acid may be watched.

Ammonium Biurate.—Ammonium biurate (see Fig. 32) is the only urate sediment which forms in an alkaline urine. It may also form while the urine is very faintly acid. It occurs with the acid urate sediment after the reaction changes; also with amorphous phosphates and triple phosphates. It is formed as ammonia increases in the urine. Microscopically it is a beautiful sight, the spheres often presenting the so-called " morning star" shape or " thorn-apple" crystals. These are spheres of a dark color, often concentric or radially striated, and have on their surface thorns,. More often these spheres have long projections, giving them very bizarre shapes. They are soluble in acetic acid with the subsequent precipitation of uric acid, giving off ammonia.

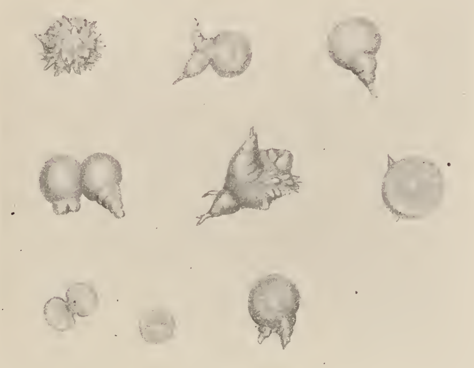


Fig. 32.—Various forms of ammonium biurate crystals. × 400.

Another very rare form resembling phenylglucosazon sheaves is pictured in Fig. 33. These sheaves of very large coarse yellow needles, since they occurred with the ordinary forms of ammonium urate and were so soluble in acetic acid, were perhaps this salt. Note their coarse size. (Dr. Boggs has shown this sediment to be calcium phosphate.)

The yellow color of these urate sediments is due to urochrome especially, also urobilin. The red is due to uroerythrin. This sediment may contain all the bile that there is in the urine and much of the black pigment in case of carbolic acid poisoning.

Crystals of sodium biurate are rare, and occur in urines undergoing ammoniacal decomposition but yet amphoteric. These crystals resemble calcium phosphate, but are soluble in acetic acid, which gives at once a cloud of uric acid crystals.

Uric Acid.—Uric acid (see Fig. 34) when pure crystallizes usually in rhombs, but in the urine the corners are dissolved, giving the so-called " whetstone" crystals. When seen on the edge these crystals are

very narrow rectangles. They may be single or in rosettes, or clustered in the shape of a barrel (see Fig. 34, a, b, 1). Their color is from a yellow to a brown, or they may be colorless. The colorless crystals are sometimes perfect hexagons (see Fig. 42, e), in which case their recognition is difficult, since they resemble cystin perfectly. A recent case of Dr. Futcher's, which urine he kindly gave me for demonstration, illustrates this point. The patient was a girl six years of age, with diabetes mellitus. The urine was 1000 to 2000 cc. in amount, specific gravity about 1035, and sugar 5.1 to 5.5 per cent.; nothing of interest microscopically. After twenty-four hours on a carbohydrate-free diet the urine was turbid, showing a suspension of glistening particles (sp. gr. 1026; sugar 0.6 per cent.). Microscopically, the turbidity was seen to be due to colorless hexagonal crystals almost exactly resembling cystin, many single, most in clumps of even macroscopic size. It was only after testing chemically that they were recognized as uric acid. On this day no typical uric acid crystals were seen. The following day there was a mixture of hexagonal and whetstone crystals, which former later disappeared.

Some are in needles arranged in sheaves (see Fig. 34, 4).

Their color is due to urochrome, not to urobilin, and the red is due to uroerythrin plus urochrome. Urobilin, hæmatoporphyrin, bilirubin, or biliverdin may give the color to the crystals. In cases of carbolic acid poisoning these crystals are a dark brown, almost black color. These crystals may occur in masses as large as the head of a pin, which cling to the glass (see Fig. 34, 2).

If these crystals are precipitated artificially by acid they are of a reddish-brown color due to black decomposition products of urochrome; they may be stained by indigo-blue or indigo-red.

Calcium urate crystals are said to sometimes occur with calcium oxalate; they are colorless prismatic crystals, insoluble in hot water, give the murexid test, and if acid be added uric acid crystals are deposited. They may be produced by treating the urate sediment with lime water.

Detection.—In acid urine the urate sediments may usually be recognized from their gross appearance, but particularly from the fact that they disappear on warming and that all are dissolved by acid with the subsequent precipitation of uric acid. Uric acid itself is not dissolved by heat or acid. Ammonium biurate is soluble in acid, and uric acid crystals then appear. The spheres are of characteristic form.

Murexid Test.—The crystal or sediment is evaporated in a porcelain dish with dilute HNO_3. To the residue is added weak NH_4OH. A beautiful purple-red color is obtained.

The significance of the urate sediment is very slight, it depending chiefly on the concentration and the acidity of the urine. Uric acid,

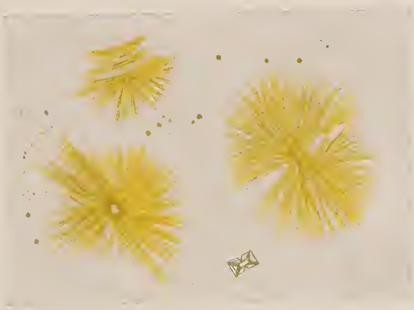


FIG. 33.—Sheaves of ammonium urate (?) needles. × 50.

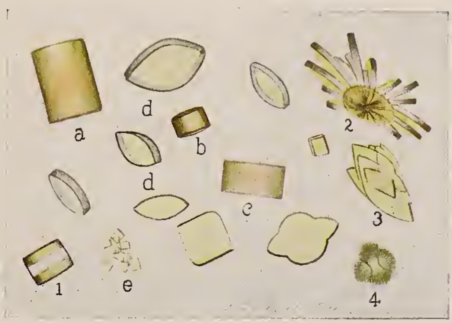


Fig. 34.—Uric acid crystals. (The lettered forms are drawn from nature, the figures copied from Rieder's Atlas.)

however, is somewhat more important since it may form large concretions.

Phosphates and Carbonates.—(1) AMORPHOUS EARTHY PHOSPHATES AND CARBONATES may be precipitated in any urine by the addition of a little fixed alkali. A somewhat similar precipitate forms upon heating a weakly acid or alkaline urine for albumin, since the acid salts are changed to insoluble basic ones. Both are soluble in acetic acid, the carbonates with gas evolution. They are the chief constituent of the sediment of an alkaline urine, and may be present in fresh urine in cases in which much acid is lost from the stomach, as in hypersecretion with vomiting or treated by lavage, or if diarrhœa be present. In the so-called phosphaturia, however, the total amount of phosphoric acid is not increased. Microscopically, this precipitate appears as very coarse colorless granules varying considerably in size, which disappear on the addition of a little acetic acid. By the gas formed it may be seen which granules were carbonates.

FIG. 35.—Various forms of triple phosphate crystals. × 400. To the left are coffin-lid shapes; in the lower centre a perfect pryamid; that in the upper left corner resembles neutral magnesium phosphate; that in the upper right is a partially dissolved crystal.

(2) TRIPLE PHOSPHATES, $MgNH_4PO_46H_2O$.—These beautiful crystals (see Fig. 35) appear in urine even still acid as soon as sufficient ammonia is present to form them. They accompany usually the amorphous carbonates and phosphates, and often ammonium urate, and may be the chief constituent of the sediment. They belong to the rhombic system, and vary in size from those very small to some even 9 mm. in length. Of their shapes, the so-called coffin-lid crystals are characteristic (see Fig. 35). They are often very irregular and of a great variety of shapes, due to rapid crystallization from a concentrated solution, or especially as they become partially dissolved, leaving X-forms. Some are said to resemble calcium oxalate crystals, but we doubt this, since even when perfect pyramids with square base the difference is apparent (see Fig. 35).

Fern-shaped crystals occur especially in sediments artificially precipitated.

In some urines these crystals are nearly all of unusual shapes,—very thin plates (see Fig. 35), some with bevelled edges, some apparently not; some with square, some rounded or bevelled corners; some are wedges (see Fig. 36), some triangular prisms; yet all give by refraction a greenish hue which is not seen in the calcium oxalate.

Neutral Magnesium Phosphate, $Mg_3(PO_4)_2 22H_2O$.—These very rare crystals (see Fig. 42, b) occur in alkaline urines in which not sufficient ammonia is present to form the above. Such is the case in certain cases of dilated stomach with considerable vomiting, and also after the ingestion of magnesium carbonate, etc. These crystals are exceedingly refractile, long rhombic tablets with bevelled edges. They form a beautiful sediment. Some resemble the very thin coffin-lid triple phosphate crystals (see Fig. 35).

Dicalcium Phosphate.—These crystals form in amphoteric or weakly acid urine. They are rare. They appear as small prisms or

Fig. 36.—Atypical forms of triple phosphate crystals. × 400.

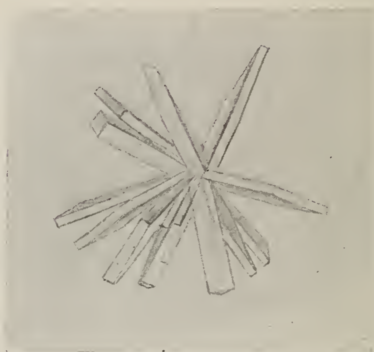


Fig. 37.—Wedges of dicalcium phosphate.

wedges in irregular clumps (see Fig. 37), or are massed together in rosettes (see Fig. 42, d) or fan-shaped clusters. These masses or rosettes are usually so thick that the individual small crystal can hardly be made out. A rather unusual form of probably calcium phosphate is shown in Fig. 38. They occur when the urine is rich in calcium and only weakly acid; among diseases, especially in joint troubles. They are soluble in acetic acid. They may be separated from triple phosphates, since 20 per cent. ammonium carbonate will dissolve these and not the latter.

Calcium Carbonate.—These crystals (see Fig. 39) may be mingled with the amorphous carbonates in an alkaline urine. They occur as amorphous masses or as dumb-bells a little like CaOx, or large concentric radiating spheres. They are soluble in acetic acid with gas formation.

NEUTRAL CALCIUM PHOSPHATE also forms a scum on the surface of the urine, even when quite fresh, giving the appearance of a film of oil, and which may be easily skimmed off. This consists of an amorphous precipitate which under the microscope resembles sheets, often seen when one is not careful always to wipe off the outside of a pipette before making a preparation for microscopic examination.

Oxaluria.—This symptom complex, formerly so respected, has fallen into disrepute. The old criterion for its presence was a large sediment of CaOx crystals, but this sedimentation does not depend so much on the total amount of oxalic acid present as it does on its solubility. Yet it is of much practical importance, since CaOx occurs so often in calculi, in even 30 to 50 per cent. of them, and these are the worst stones. The chief source of the CaOx is the food, certain vegetables, as beans, artichokes, beets, potatoes, and especially to-

FIG. 38.—Calcium phosphate (?). × 400.

matoes, spinach, rhubarb, certain fruits and grains, cocoa, tea, coffee being particularly rich. The most ingested is destroyed in the intestine, only 15 per cent. of the oxalic acid being absorbed: this is dissolved by the HCl in the stomach and excreted quantitatively as CaOx; about 10 per cent. is in the stools, the rest is destroyed by the intestinal bacteria and ferments.[148]

To reduce the output, meats, fats, grains, rice, apples, pears may be allowed. These contain less calcium, the important point in preventing this precipitation. In health the output is about 20 mg. per day, with an upper limit of 35 mg. Although the most comes from the food, yet a certain amount is from tissue combustion, since some is present even in the urine of a starving person. Many consider oxalic acid a normal decomposition product of uric acid; others that glycocoll and creatin are the oxalate formers. Some may be reabsorbed from the bile. Bakhoven thinks that of the foods the carbohydrates are

[148] Klemperer and Tritschler, Berl. klin. Wochenschr., 1901, p. 1289.

the chief builders. It bears no relation to the uric acid excretion; the latter, for instance, can be increased by the nucleins, which do not affect the oxalic acid output.

Among the diseases claimed to be accompanied by oxaluria are pulmonary tuberculosis, peritoneal tuberculosis, pernicious anæmia, leukæmia, in which condition the output is claimed to be 33.2 to 53 mg. per day, jaundice, diabetes mellitus, gout, diseases of the digestive and respiratory organs, cirrhosis of the liver, and especially neurasthenia. It bears some relation to the absence of HCl in the gastric juice and to fermentation processes in the intestine. In diabetes mellitus a large output is quite surely present. This increases as the sugar diminishes (vicarious oxaluria). Naunyn mentions three cases with quantitative estimations, one with 0.8 gm., the second 1.2 gms. in twenty-four hours, the third with 0.5 gm. per litre. In the case of neurotic persons an increased output is generally granted, and v.

FIG. 39.—Calcium carbonate. × 400.

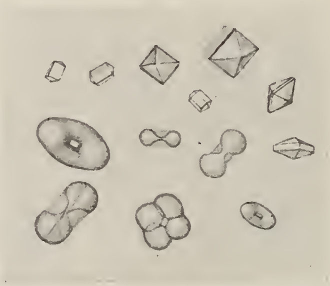


FIG. 40.—Various forms of calcium oxalate crystals and spheres. × 400.

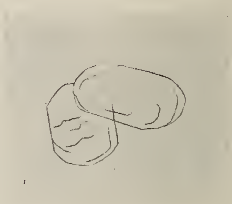


FIG. 41.—A rare form of calcium oxalate crystals.

Jaksch considers it an independent disease, since it may be the only abnormality found. The symptoms in such cases are those of neurasthenia and dyspepsia. It is of interest that insurance companies now regard "oxaluria" as an early sign of nephritis.

CALCIUM OXALATE CRYSTALS may precipitate in any urine. The cause is not fully known, but their increased presence it is hard to associate with any pathological condition. This precipitation is most important clinically. The real question is, Why is any in solution? Klemperer and Tritschler [149] consider all the acid phosphates aid in holding it in solution, the salts of sodium least, calcium more, magnesium most, and something depends on the absolute amount of CaOx. The crystals occur in two forms:

(1) The octahedral, which belong to the tetragonal system (see Fig. 40). These resemble double envelopes or prisms, and may be recognized from their appearance ($CaC_2O_43H_2O$).

[149] Zeitschr. f. klin. Med., 1902, vol. xliv. p. 337.

(2) Spheroidal forms (see Fig. 40) which are flat, oval, or nearly semicircular with a central groove; hence they resemble an hourglass. They often present a radial striation ($CaC_2O_4H_2O$).

A rare form of crystal is represented in Fig. 41, flat plates with parallel sides and rounded ends, which look like superimposed sheets of mica. In a recent case the urine had a great many of these.

These crystals are usually colorless, but may be bile-stained. They are transparent and very refractive. They are insoluble in water, very little if any in acetic acid, but easily in any mineral acid. Their crystallization probably depends on the amount of oxalic acid, on the relative amount of NaH_2PO_4 which has a greater ability in holding CaOx in solution in a warm than in a cold urine, and especially in inverse proportion to the amount of magnesium. As the precipitate forms very slowly, perfect crystals form. They may be found in acid, amphoteric, or weakly alkaline urine, and are sometimes present in the specimen when voided.

They attracted considerable attention among the older pathologists, as their irritation was supposed to explain several of the symptoms and bad habits of neurotic individuals. The shape of the octahedral forms is quite characteristic, and these cannot well be confused. Apart from their shape, their refractivity is very suggestive, and it is only on hasty examination that they could be mistaken for triple phosphate crystals, even when the latter are square and perfect, but single, pyramids. They may also be easily separated from these by their insolubility in acetic acid. The spherical forms could be mistaken for $CaCO_3$, but these are soluble in acid with gas production and show a different structure.

Quantitative Determination of Oxalic Acid.—The Neumann's method is as follows: The twenty-four hours' amount of urine is precipitated with calcium chloride and ammonia, and then acetic acid is added until a weak acid reaction. A small amount of alcohol thymol solution is then added to inhibit bacterial growth. The precipitate after long standing, over twenty-four hours in a warm place, is washed several times by decantation, pouring the fluid through the filter, then the precipitate brought onto the paper. Wash as much as possible by decantation, since the fine precipitate easily passes through the paper. The precipitate is then dissolved in somewhat warmed dilute HCl, and the paper washed with water until the acid reaction disappears. The filtrate is evaporated in a porcelain dish on the water-bath to a small volume. The fluid is then placed in a small stout cylinder, the dish being washed with water and dilute HCl, and the wash-water added to the fluid. Ammonia is then added in excess and the whole stained with a few drops of litmus, to be sure of the reaction. After long standing, at least twenty-four hours, the precipitate is brought onto an ashless filter paper. It is necessary to remove the crystals from the walls of the cylinder by rubbing well with a glass rod protected with a small piece of rubber tubing. The precipitate is then washed with water until it is chlorine-free, and then with acetic acid. The filter is then dried, burned in a platinum crucible at a dull red, then heated with a blast flame until at constant weight. Calcium oxalate is thus transformed to calcium oxide, 50 parts of which correspond to 90 parts of oxalic acid.

Calcium Sulphate, $CaSO_4 2H_2O$.—This is a very rare sediment occurring in very acid urines. The crystals (see Fig. 42, a) are long and thin tablets or needles, single, but more often in clusters, which are insoluble in NH_4OH, alcohol, and acetic acid. They are difficultly soluble in HCl, HNO_3, and hot water. They are more soluble in hot water than in cold. The solution should be tested with $BaCl_2$, to make sure of sulphuric acid.

Hippuric Acid.—This acid occurs rarely as a sediment, as milk-white, semi-transparent, four-sided prisms and rods with ends of two to four planes (see Fig. 42, c). These are distinguished from uric acid, which they may resemble in form, by their greater solubility in water, especially in warm, their solubility in alcohol and ether, and that they do not give the murexid test. The normal amount of hippuric acid in the urine is from 0.1 to 1 gm. per day, and varies as the diet.

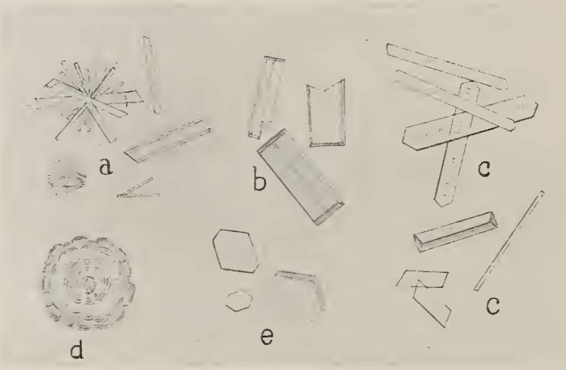


Fig. 42.—Various crystals. a, calcium sulphate; b. neutral magnesium phosphate; c, hippuric acid; d, acid calcium phosphate; e, colorless uric acid. (Copied from Rieder's Atlas.)

Hetero-albumose.—In two cases it has been found in the sediment, once crystalline and once amorphous.

Xanthin.—Two or three cases have been reported in which xanthin crystals appeared in the sediment. These resembled uric acid somewhat (see Fig. 43, d), but are soluble on heating and in ammonia. They are evaporated in quite concentrated HNO_3 on a bath, and give a yellow residue. On careful heating further over a small flame this becomes intensely yellow, and if KOH be added, yellowish-red. Warmed further, it becomes a deeper red, even a violet-red. This is not the murexid test, and should not be confused with it.

Hæmatoidin (Bilirubin).—These crystals appear as needles (Fig. 43, a) or rhombs (b) in the sediment, sometimes in hæmorrhagic nephritis, and in very jaundiced urine especially after acid is added, in acute yellow atrophy and in fragments from cancers. They also occur in pyonephrosis and after transfusion. In the jaundice of the newborn they occur in the epithelial cells of the urine. They have also been found in waxy kidney, scarlet fever, typhoid fever, and carcinoma of the liver with jaundice. They also occur in amorphous form.

Indigo.—The crystals of indigo may occur in normal decomposing urine as a scum of blue needles arranged in stars, or blue rhombic plates, soluble in chloroform to blue solution. These are more often seen in the decomposing urine of peritonitis, pyelonephritis, etc. One also sees violet-red bundles of crystals or plates of indigo-red.

Melanin has been found rarely as amorphous scales.

Hæmoglobin occurs in cases of hæmoglobinuria as amorphous scales, plates, or casts.

Cholesterin.— Cholesterin sometimes occurs in flat superimposed plates, often with re-entrant angles (see p. 625), in such amounts as to justify the term "cholesterinuria." It is always found in association with other fats. This may occur in vesical catarrh, especially in pyelitis, pyonephrosis, echinococcus cysts of the kidney, and nephritis. The crystals are also formed from the fatty degeneration of pus-cells and of breaking-down tissue. It is rare from fatty degeneration of the kidneys, and does not occur in chyluria, in which case one would

FIG. 43.—Various crystals of the urine. a, hæmatoidin needles; b, hæmatoidin crystals; c, leucin; d, xanthin; e, tyrosin. (Copied from various authors.)

expect it. Hirschlaff[150] reports a case of hydronephrosis (thought to be due to a stone and with the emptying of a large sack) with even 5.8 gms. of cholesterin in 100 cc. of urine. We had a case of long standing cholesterinuria of considerable degree in a case of renal cyst of doubtful nature.

Cholesterin is insoluble in cold alcohol, but easily in hot, reprecipitating on cooling, and is soluble in chloroform. If the cholesterin solution be superimposed on concentrated H_2SO_4, the former solution is first blood-red, then more violet-red, while the sulphuric acid becomes dark red with green fluorescence (Salkowski). If the crystals microscopically be brought into contact with H_2SO_4 4 parts, H_2O 1 part, this play of colors can be watched.

Leucin and Tyrosin.—Leucin and tyrosin occur in the urine in certain pathological conditions. As a spontaneous sediment leucin does not occur, while tyrosin has been found in very few (three) cases, one

[150] Deutsches Arch. f. klin. Med., 1899, vol. lxii. p. 531.

of which was of acute yellow atrophy, one of phosphorus poisoning. These bodies may often be found in solution in acute yellow atrophy, phosphorus poisoning, rarely in smallpox, severe typhoid fever, pernicious anæmia and leukæmia. With the exception of the few cases in which the tyrosin sheaves have been found in the unconcentrated urine, it is necessary to evaporate the urine to about one-tenth its volume. The addition, then, of alcohol will usually give a sedimentation of needles of tyrosin and spheres of leucin; peptone and lactic acid are also present. The needles of tyrosin are black in appearance and are grouped together like sheaves of wheat (see Fig. 43, e). Since jaundice also occurs in practically all cases, the crystals of bilirubin must be excluded; these have an intense brown color, but in some cases a rather similar shape. In an alkaline urine the calcium phosphate must be excluded. LEUCIN, if pure, is in groups of spherules (see Fig. 43, c) which have little refractility and hence differ from the urates. They have a much clearer contour and no spicules, and a hyaline or a radiating structure. Their appearance varies, however, with their purity, and if impure they may be in spheres or masses with no hyaline structure whatever. They may have a dark centre and a clear periphery, or *vice versa*.

The microscopical diagnosis of these bodies is almost never sufficient, but should be confirmed by chemical tests. In so doing it is quite necessary to use a fresh urine, since these bodies rapidly and easily form in a decomposing albuminous urine, hence in an old urine the question is whether they are preformed or not.

In all tests it is necessary, first, to remove the albumin by heat and acid and examine the filtrate. This is first precipitated with neutral, then with basic, lead acetate until all precipitation ceases. The urine is then filtered, the lead removed with H_2S, the filtrate concentrated by evaporation. The tyrosin even now separates out slowly if in considerable amount. The concentration should be carried on to very small volume, and the urea extracted by absolute alcohol. The residue is then boiled with weak ammoniacal alcohol and the filtrate is again evaporated to small volume and then allowed to crystallize. The leucin or the tyrosin will separate out, that one first which first becomes saturated. The partial separation may be obtained with alcohol in small volume which dissolves the leucin more easily than the tyrosin. If no precipitate appears, again dilute and precipitate with basic lead acetate and repeat.

A better separation of leucin and tyrosin is the following. The residue after evaporation is dissolved in boiling water plus a little ammonia. To the hot solution is added basic lead acetate, stirring all the while until the precipitate is no longer brown but white. It is then filtered, heated nearly to boiling, made slightly acid with dilute H_2SO_4, and then boiled to drive off the ammonia and to pre-

cipitate the lead. It is then rapidly filtered and cooled. The tyrosin will precipitate almost quantitatively. To the solution is added H_2S to precipitate the lead, and it is evaporated to smaller volume. While boiling $Cu(OH)_2$ freshly precipitated is added in excess and the boiling continued for a few minutes. The precipitate will contain part of the leucin. This precipitate is suspended in boiling water, decomposed with H_2S, and a little acetic acid added. It is then filtered. The filtrate is decolorized with animal charcoal and evaporated to small volume. On cooling the leucin will separate out. The rest of this body will be in the blue copper compound. It is very hard to get leucin pure, although it can be done by forming its ethyl ester.

Tyrosin, $C_6H_4CH_2CHNH_2COOH$.—Tyrosin crystals (see Fig. 43, e) precipitate from water solutions in bundles of needles arranged like sheaves of wheat, from ammoniacal alcohol in bunches of prisms. These are soluble in water, slightly in alcohol, not at all in ether, and easily in acids and alkalies. Its crystalline shapes are not characteristic. The sediment should be filtered out, washed with water, dissolved with ammonia plus a little ammonium carbonate in warm solution, and evaporated until it recrystallizes. The chemical tests cannot be made directly in the urine.

Piria's Test.—Some dry tyrosin is placed in a test-tube and a few drops of concentrated H_2SO_4 added. This is warmed gently and then boiled in a water-bath for half an hour. A red solution of tyrosin sulphate is obtained. The solution is cooled. To it should be added several volumes of water, washing the tube well, it neutralized with $BaCO_3$, and filtered. The filtrate is evaporated to a few cubic centimetres, and weak Fe_2Cl_6 is then added (acid-free) to the cooled solution. A fine violet color results. This test is prevented by free mineral acids or an excess of Fe_2Cl_6.

The hot aqueous solution of tyrosin gives, with Millon's reagent, $(Hg(NO_3)_2+KNO_2)$, while hot a fine red color, and an abundant red precipitate.

Leucin $(CH_3)_2CHCH_2CHNH_2COOH$.—Leucin is present as spherules; their color and regularity of outline depend on the purity of the specimen. Often daughter spherules project from them, and they frequently show a striation. Leucin (see Fig. 43, c) is soluble in water, less in alcohol, and very in acids and alkalies. All of these compounds are more soluble in an impure than in a pure condition. None should ever be expected in a sediment until the urine is concentrated. It is isolated by the above methods. For the chemical tests it must first be purified by recrystallizing from hot ammoniacal alcohol. The characteristic tests are its crystallized form when pure, the fact that it sublimes at a gentle heat at 170° C. without fusion to a woolly mass and with the odor of amylamine.

Scherer's Test.—Pure leucin plus a little HNO_3 is evaporated on a platinum foil. A colored residue is obtained. The slight residue is warmed with NaOH, and a water-clear, if pure, or colored if im-

pure, fluid results. This is evaporated carefully and an oily fluid obtained which rolls around without wetting the foil. This test is characteristic for even impure leucin.

SALKOWSKI'S TEST.—To the specimen is added a little water plus one or two drops of 10 per cent. $CuSO_4$. A blue solution is obtained, $(C_6H_{12}NO_2)_2Cu$, which does not reduce on heating.

Cystin.—This rare condition (only 131 cases now reported in literature. Simon and Campbell), which occurs in certain persons perhaps during their whole life, is accompanied by no symptoms except those from the calculi formed; some persons undergo repeated operations because of these stones, and live a life of misery. The formation of calculi, however, is intermittent, and after a period of misery the

FIG. 44.—Cystin crystals from urine. × 400.

person may for a long time be free. The output of cystin is in some cases intermittent.

Cystin is a normal intermediate product of normal proteid metabolism, the sulphur portion of the proteid being for the most part, possibly all, in this radical. It is not normal in the urine; if fed to a normal person, about 66 per cent. of its sulphur is excreted as sulphates and about one-third as neutral sulphur; none as cystin. Simon and Campbell[151] think some is eliminated in the bile as taurocholic acid. Why it should be excreted is not known. There are two general theories,—the one that it is the product of an intestinal mycosis, which is borne out by the fact that both intestinal contents and urine contain certain diamines, as cadaverin, putrescin, and others; the other, that it is an individual variation in metabolism, an inability on the part of the organism to oxidize the cystin nucleus.

In the urine while fresh are seen hexagonal transparent crystals of cystin. These crystals (see Fig. 44) are very characteristic, yet not absolutely so, since in certain cases the uric acid may assume this form. Sometimes large concretions form, from a pin-head size to 1 cm. in diameter, which are rather soft and waxy, crystalline on cross-section, and are of a whitish-yellow color. These crystals are soluble in ammonia and reprecipitated by acetic acid, a test which must be applied to exclude uric acid.

[151] Johns Hopkins Hosp. Bull., 1904.

We have seen but four cases. One of these patients has on many occasions for some years had these stones crushed. Another case, a woman, was distressed for years by these concretions, but refused operation, and as she has since then attained considerable success in public life, we presume that the stones no longer bother her so much.

The urine in such cases on standing often gives the odor of H_2S. It is in this condition particularly that the neutral sulphur of the urine is largely increased, and the neutral sulphur is the best index of the amount of cystin present.

The presence of **diamines** in the urine and in the fæces in traces has attracted some attention.[152]

There occurs sometimes putrescin, sometimes cadaverin, sometimes both, and their presence is variable and intermittent. Lewis and Simon, in 1902, stated that they had been found in seven cases.

Baumann's method for their detection is as follows: The twenty-four hour amount of urine is shaken up with 10 per cent. NaOH and benzoylchloride (in the proportion of 1500:200:25) until the odor of benzoylchloride is gone.

The precipitate (of phosphates, carbohydrates, and benzoylated diamines) is filtered with the aid of a suction-pump. The precipitate is digested with alcohol, filtered, the extract evaporated to small volume, 30 volumes of water added, and allowed to stand twelve to forty-eight hours. The benzoylated diamines separate out in the milky fluid as a voluminous sediment of white crystals. This is redissolved in alcohol, concentrated to small volume, and diluted again with water. This is repeated several times to separate the carbohydrates.

From the first filtrate more may be recovered by acidifying with H_2SO_4 and extracting three times with ether. To the ether residue is added 12 per cent. NaOH till neutral, then 3 to 4 volumes of the alkali. This is then kept in a cold place for crystallization and crystals of cystin and the diamines will separate. They are filtered and suspended in cold water; the benzoylchloride crystals remain.

The crystals are dissolved in a little warm alcohol, then 20 volumes of ether added; benzoylputrescin is precipitated, melting point 175° to 176° C. The ether residue contains benzoylcadaverin, melting point 129° to 130° C.

Unorganized Sediments.— The following outline for use in recognizing an unorganized sediment is so useful that we quote it in full as given by Neubauer and Vogel.

A. Acid urine.

(*a*) Sediment amorphous.

(1) Sediment consists of fine granules in clumps, mingled with which are crystals of uric acid and calcium oxalate; *urate sediment.* This sediment is soluble on warming, and if a drop of strong acetic acid be added the granules gradually disappear with the subsequent separation in a few hours of uric acid crystals.

(2) Dumb-bell shaped bodies.

(*a′*) Insoluble in strong acetic acid, soluble in concentrated hydrochloric acid without subsequent crystallization; *calcium oxalate.*

[152] Simon, Am. Jour. Med. Sci., 1900, vol. cxix. p. 39; 1902, vol. cxxiii. p. 838; Schollberg and Garrod, Lancet, August 24, 1901.

(*b′*) Insoluble in concentrated hydrochloric acid; probably *calcium sulphate.* The sediment should be filtered, washed, dissolved in much hot water, and tested for calcium and sulphuric acid.

(3) Very refractive globules, soluble in ether; *fat.*

(4) Amorphous yellow granular masses: bilirubin or *hæmatoidin.*

(*b*) Sediment crystalline.

(1) Yellow or brown whetstone-shaped crystals, single or rosettes, alone or with amorphous urates and calcium oxalate: *uric acid.* These crystals are soluble in sodium hydroxide, then with the addition of concentrated hydrochloric acid a reprecipitation of uric acid crystals.

(2) Small yellow rhombic tablets alone or with amorphous granular tablets of the same color, often embedded in tissue detritus: *bilirubin* or *hæmatoidin.*

(3) Colorless (or yellow in a decomposed urine), transparent, strongly refractive octahedrons, or double envelope forms, or quadrangular short and narrow prisms with octahedrons at the ends, insoluble in acetic acid, soluble in hydrochloric acid: *calcium oxalate.*

(4) Crystals somewhat similar to the last mentioned, or large coffin-lid crystals, soluble in acetic acid: *ammonium magnesium phosphate (triple phosphates).*

(5) Symmetrical hexagonal tablets, sides and angle almost equal, insoluble in acetic acid, soluble in ammonia: *cystin.*

(6) Colorless whetstone-shaped tablets, insoluble in acetic acid; soluble in ammonia. On the addition of hydrochloric acid to this solution hexagonal tablets separate: *xanthin.*

(7) Large, flat, strongly refractive elongated rhombic tablets, soluble in acetic acid, and partially in ammonium carbonate: *normal magnesium phosphate.*

(8) Prisms, single or in rosettes,

(*a′*) Soluble in ammonia: *hippuric acid.*

(*b′*) Insoluble in ammonia and in acids: *calcium sulphate.*

(9) Wedge-shaped prisms, single or in clusters, or in thick rosettes, which are decomposed by ammonium carbonate, and soluble in acetic acid: *acid calcium phosphate.*

(10) Bunches of very fine needles insoluble in acetic acid, soluble in ammonia and hydrochloric acid: *tyrosin.*

B. The urine alkaline when the crystal precipitates. (After the urine becomes alkaline many of the sediments previously mentioned which separate in the acid urine may still remain.)

Amorphous.

(1) Small granules together with triple phosphate crystals,

(*a′*) Soluble in acetic acid without gas formation: *normal phosphates of the alkaline earths.*

(*b'*) Soluble, but with gas formation: *carbonates of the alkaline earths.*

(2) Dumb-bell shaped masses or large spheres, soluble in acetic acid with gas formation: *calcium carbonate.*

(3) Large dark spheres often covered by small projecting crystals: *ammonium urate,* soluble in hydrochloric acid or acetic acid with the subsequent separation of the rhombic crystals of uric acid.

Crystalline.

(1) Large colorless prisms, many coffin-lid shaped: *triple phosphates,* soluble easily in acetic acid.

(2) Rosettes of very fine blue needles or blue tablets: *indigo.*

(3) Rosettes of violet-red needles or rhombic platelets: *indigo-red.*

Chyluria.—Chyluria differs from lipuria in its gross characteristics, the term being used of a urine which resembles an emulsion of fat, hence like dilute milk. When less fat is present and the gross appearance not so striking, the term *lipuria* is used.

In chyluria considerable fat is present. This may form gross tallow-like masses, but as a rule the particles are microscopically much finer than in milk, even on the limits of visibility. The urine appears like a thin milk, and sometimes has a reddish tinge of blood, in other cases a whey-like appearance. Fresh, the urine is weakly acid or neutral, and does not have the normal urinary odor. On standing often a cream arises or a fibrin coagulum forms. In addition to the fat the urine contains always albumin, sometimes cholesterin and lecithin. The proteids found are serum globulin and albumin. Fibrogenic substances have also been found, hemialbumose and peptone. The proteid may be present from 0.2 to 2 per cent. or more, and the fat from a trace to 3 per cent. A few leucocytes may be present and a few red blood-cells. In parasitic chyluria one finds the eggs and embryos of Filaria usually in coagula. Casts, etc., are always absent unless a complicating Bright's disease is present. The urine may be chylous during the night, and clear during the day, or *vice versa.* In other cases the excretion of the fat is dependent upon the position of the patient, occurring only when in a vertical position, after digestion, bodily exercise, or excitement. In certain cases the coagula formed in the bladder have caused considerable trouble. It is a disease which lasts from months to years, often with intermissions. It may cease spontaneously. This disease occurs endemic in the tropical and subtropical regions, in some cases in the temperate zone.

There are two forms, that due to the filaria, and the non-parasitic form the etiology of which is not understood.

Concerning the latter, some say that sugar is not present in the urine, and were it simply lymph present it certainly would be; also

that there is a higher percentage of fat in the urine than occurs in the lymph. Again, there is no decrease in the percentage of the normal urine constituents. In some cases a fat diet will increase the chyluria, and even a foreign fat may be recognized. The theory of Claude Bernhard was that chyluria was the result of an abnormal fat content of the blood due to poor assimilation; but an increase of the fat of the blood is very rare, and this does not explain the albumin found in the urine. Others think it due to liver disease. It can only be said that there is no severe renal lesion to explain it. Franz and Styskal give these as reasons why this fat is from the lymphatics; that it disappears on starvation or fat-free diet, foreign fat may be recognized, it appears early in alimentary glycosuria, and small mononuclear cells are present.

Lipuria.—As has been said above, this differs from chyluria in its gross appearance. It is a condition which is often reported in the hospitals, but by beginners who have not excluded oil in the catheterized specimens of urine. Again, the microscopical appearance is not always sufficient. It should be tested chemically, the urine extracted with ether, and the residue examined. This heated gives the odor of acrolein. The residue also will make a fat-spot on paper, and will give the osmic acid test.

Also to be excluded are, fat from the rectum, deception, the tenacious phosphate sediment, and the scum of bacteria forming at the top of the urine. Normally there is microscopically little if any fat in the urine, and of this the source is the blood. Lipuria may result from an over-ingestion of fat in the diet or as a medicine (cod-liver oil), the so-called "alimentary lipuria;" from the subcutaneous injection of oil or oil rubbed into the skin; the fat may come from various organs, especially after fractures of bones if the marrow be crushed; rarely after inflammation of the marrow; in eclampsia, which disease was formerly supposed to be due to the crushing of the fat of the pelvis of the kidney; crushing or tearing of the subcutaneous fat, of the liver, or of fatty tumors; among diseases are, diabetes mellitus, alcoholism, tuberculosis, adiposity, nephritis, certain mental diseases, pancreatic diseases, cardiac diseases; after various protoplasmic poisons. In the last mentioned group there may be an increase of fat in the blood, but this needs confirmation. The relation of the lipuria to lipæmia has been proved for fractured bones, subcutaneous bruises, and diabetes mellitus. In the diseases of the urinary organs a slight grade of fatty degeneration of the kidneys may explain the condition, which occurs in nephritis, various infections, intoxications, anæmias, and cachexias. The fat may also arise from the fatty degeneration of epithelial cells, leucocytes, casts, and fragments of tumors; in such cases the most of the fat remains in the cells, or collects in droplets which float on the surface.

Organized Sediments. Mucous Sediment.—The "nubecula" is a very faint cloud of mucous strands appearing soon after the urine cools, which first collects at the top, then sinks to the bottom of the glass. It is mucus from the epithelial cells of the urinary passages. These strands enclose a few "mucous corpuscles," mononuclear or polymorphonuclear leucocytes often some amœboid, and some crystals. When much mucus is present in the urine it may form a translucent or cloudy coagulum-like sediment which is more clearly seen after the addition of acetic acid. This is the product of desquamatory catarrh of the mucosa.

Epithelial Cells.—It is normal for a few cells to be present in the nubecula, since the mucosa of the urinary passages is an epithelial surface, hence is always desquamating somewhat. These cells are increased in inflammatory and destructive lesions, in which case cells

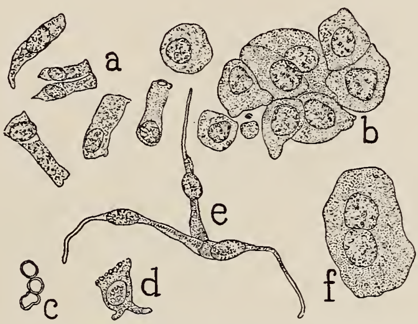


FIG. 45.—a, e, d, cells from male urethra; b, f, cells from transitional epithelium; c, shadows of red blood-cells. × 400.

from the lower layers, which are normally never present, may also appear.

RENAL EPITHELIAL CELLS.—The cells from the kidney (see Fig. 47, e) are round or cubical, a little larger than leucocytes (12 to 25 microns) from which they are distinguished by their size and nucleus. The latter is large and vesicular, especially well seen if stained. The protoplasm is nearly always fatty, either finely so, or so very fatty that it may resemble a colostrum corpuscle (c, h). These cells sometimes show definite myelin degeneration and free myelin globules similar to those in the sputum (see Fig. 47, d).

These are very rare in normal urine, occur in any form of nephritis, but especially the acute parenchymatous, singly, in clumps, or attached to casts, and in renal infection in masses of pus-cells (see Fig. 47).

EPITHELIAL CELLS FROM THE URINARY PASSAGES (see Fig. 46, b, c, d, and Fig. 45, b, f).—These cells may be large and irregular,

round or polygonal; they are flat, with clear protoplasm and usually with a small, very distinct central nucleus. Their edges are sometimes very refractive, thin, and horny. These are the typical pavement cells from the superficial layers of transitional epithelium. In cases in which they are largely increased, as, for instance, the result of too strong irrigating fluids, they may occur in large sheets. Dawson[153] found that these cells varied in size and shape, some being irregular, large, and polygonal, some smaller and hexagonal, the larger often having a peripheral non-granular zone. The nucleus was round or oval, sharply defined and central, and many were budding.

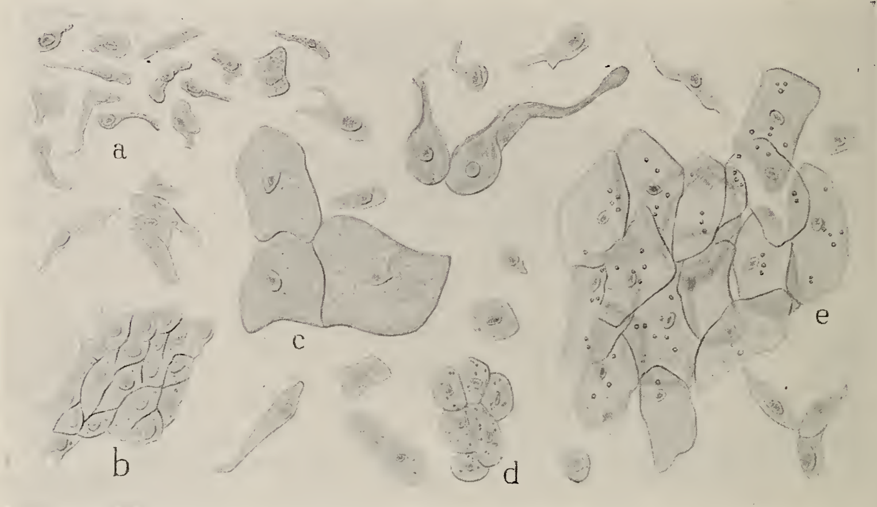


FIG. 46.—Various forms of epithelium cells in the urine. a, "tailed" cells; b, d, small polygonal; c, large surface cells; to the right of d is a small round cell of uncertain origin; e, squamous cells from vagina. All of these cells except e were obtained by ureteral catheterization, hence from the pelvis of the kidney or scraped from the mucosa of the ureter. The latter is especially true of b, c, d, and neighboring cells, which are the forms one gets from normal cases. a were from cases of pyelitis. × 400.

Among these cells were large giant-cells with fifteen nuclei. In no cells did he see the cupping of the under surface supposed to be present.

The flat polygonal SQUAMOUS EPITHELIAL CELLS (see Fig. 46, e) from the prepuce, end of the ureter, vagina, and fossa navicularis, cannot always be distinguished from the superficial cells from the bladder, although usually the stratified grouping of those from the vagina makes diagnosis easy.

The CYLINDRICAL CELLS (see Fig. 45, a, e, d) of the urethra are longer, bluntly pointed, and smaller than the above, and occur in pairs or clusters.

[153] Johns Hopkins Hosp. Bull., July, 1898, p. 155.

There are found smaller polygonal or elliptical cells from the other layers of the mucosa of the bladder, ureter, and pelvis, which consist of a very granular protoplasm and a large nucleus (Fig. 46, a, b, d). Other cells are more oval, often irregularly conical, with one or two branches. Their nucleus is very distinct, their cell body long and thread-like. In addition are small round cells, with round nucleus like mononuclear leucocytes (see Figs. 46, 47), which, indeed, they may be, or cells from the deep layers of epithelium. We have seen good numbers of these round and tailed cells in the urine obtained by ureteral catheterization from normal kidney (always with some blood).

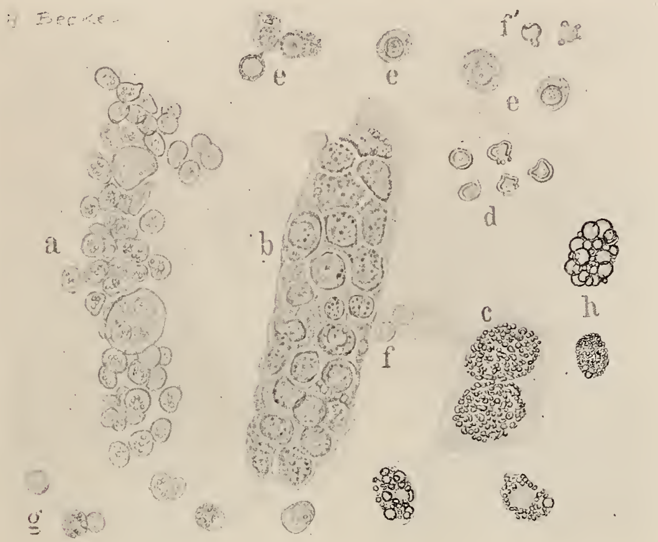


FIG. 47.—a, pseudo pus-cast; b, epithelial cast showing protoplasmic bridges between cells; c, two very granular (myelin?) renal cells; d, myelin globules; e, renal epithelial cells; f, crenated red blood-cells; g, pus-cells; h, very fatty renal epithelial cells. × 400.

These come from the middle or deeper layers of the mucosa, anywhere from the pelvis of the kidney to the bladder, singly or in clusters.

Some suppose that they can recognize the cells from the various parts of the urinary passages. Others, and this is our opinion, believe that this is usually impossible. Sahli considers that the predominance of tailed cells over flat cells indicates a pyelitis. We have seen such cases, but in a recent case of intense pyelitis, the urine obtained at autopsy from the pelvis of the kidney, the point failed. A former idea was that all tailed cells came from the pelvis of the kidney. The smaller polygonal cells from the ureter (Fig. 46, b, d) in groups are suggestive. These are the cells scraped off by the ureteral catheters.

In a recent case of streptococcus pyelitis the urine from the pelvis of the kidney showed great numbers of small round or polygonal and tailed epithelial cells in groups of considerable size, scores in each field (of 400 magnification), and of the large polygonal cells three to four in each field. Pus-cells were in great numbers; little mucus was seen.

Casts.—These have been divided into cellular, granular, and amorphous; the latter show no structure, but are homogeneous or with a faint striation. All combinations and transitions of these casts, and casts with various elements fastened to them, occur.

Epithelial Casts (Figs. 47 and 50).—These are made up of renal epithelial cells; in some cases aggregations of desquamated cells are massed together; others are certainly desquamated portions of the tubules, presenting a lumen, and having the intercellular protoplasmic bridges visible (Fig. 47). In one kidney which we have had opportunity to study, in sections of the cortex the invaginated

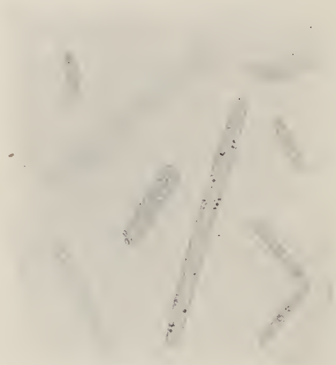


Fig. 48.—Coarsely and finely granular casts. × 400.

Fig. 49.—Waxy casts. × 400.

tubules of epithelium could be easily seen, which breaking off would give casts. Such perfect fragments are called "epithelial tubes." The cells may be well preserved, or present a marked fatty or granular degeneration. The nuclei are round and vesicular; for the recognition of the cast it is necessary to determine this point. All transitions between these and coarsely granular and fatty casts are seen.

Granular Casts (Fig. 48).—The granules may be coarse or fine. The former give to the cast a yellowish-white color. All transitions between the epithelial or leucocyte and coarsely granular casts may be found. The granules are soluble in acetic acid. To these casts may be attached epithelial cells, leucocytes, or red blood-cells. The coarsely granular casts probably represent the granular disintegration

of epithelial casts, and are formed either from epithelial tubes or from masses of cells which have previously undergone such disintegration; the outline of cells can sometimes be seen, and fat globules are commonly also present. This term often includes the hæmoglobin casts which are of a brownish red color. But another group of finely granular casts is somewhat different, and transitions between these and the opaque finely granular are not common; that is, not in the same case. These casts are covered by very fine granules, are less opaque than the coarsely granular, and fat droplets are not commonly present. When the cast is only partly finely granular the rest is hyaline; in the case of the coarsely granular it is waxy.

Fatty Casts (Fig. 50).—These striking objects are masses of fatty globules, often preserving the outlines of the original epithelial cells. They are yellowish or even blackish in appearance, soluble in

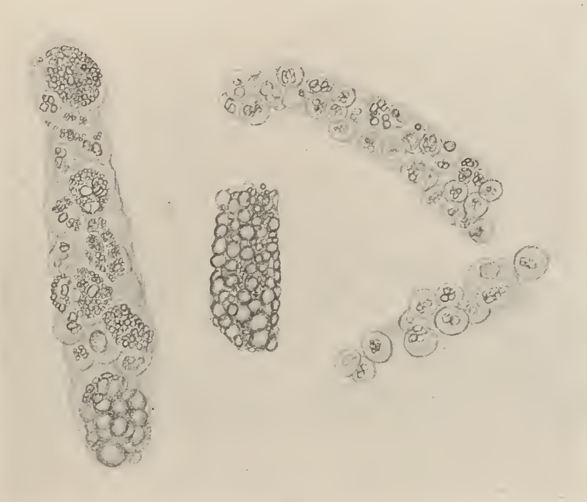


Fig. 50.—To the left an epithelial cast with very fatty cells; in the centre a fatty cast; to the right two leucocyte casts. × 400.

ether; fatty acid crystals may project from them. If any cell outline can be made out, the cast usually is called epithelial.

Waxy Casts (Fig. 49).—These casts are very refractive, sharply contoured, often of a white or yellowish color, homogeneous, and show a great tendency to split transversely, hence sometimes are in very small pieces. This appearance of great brittleness is quite characteristic. They may have any cellular elements attached. They are probably a further modification of the granular detritus of epithelial cells. Their appearance is that of wax. They are broader than the hyaline. Some give the amyloid reaction, others do not. They

are not characteristic of the amyloid degeneration, as was formerly supposed, and yet in a recent case of amyloid disease practically every cast was a waxy cast. In general there are two very distinct forms of waxy casts,—the yellow and the blue. The former were often called fibrin casts; they resemble beeswax, the latter paraffin. These occur in any nephritis with granular casts, especially when the urine is diminished, or just before death.

In the urine obtained just before death one may see the most beautiful waxy casts. In one such case recently there were many granular and waxy, no hyaline. The casts were enormous, many granular being 0.136 mm., and waxy 0.102 mm. in diameter. The latter looked as if cut out of paraffin. But this is not always the case. In another specimen only hyaline casts were present, no waxy. But these casts were not typical hyalines, yet they were not at all refractive. In other cases all forms may be found. The great difference between hyaline and waxy casts is their refractility, and it is hard to believe that they are not directly related.

Between this group and the next is a very large group of casts, the commonest form in some cases of nephritis, which have not the physical properties of wax, yet are more distinct and solid-looking than the hyaline, which name they bear. It is important to recognize that some of these have deposited on them fine granules from the urine, giving them the appearance of the finely granular casts. Their chief difference from waxy casts is that they are not so solid-looking and do not give the same color tests.

Hyaline Casts, Colloid or Glassy Casts (see Fig. 51).—These are pale, very little refractive, watery in appearance, and difficult to see unless the light is almost shut off, or unless crystals or cells are attached to them. It is advised to stain them with Lugol's, giving them a yellow color, or aniline-violet, giving blue. They give the microchemical tests for albumin. They may have the same cells attached as the above mentioned casts. Their outline is very regular. These casts, which occur in circulatory disturbances where there is no question of inflammation, are so different in appearance from the hyaline casts just mentioned that they deserve a separate name. They are soluble in acetic acid.

Blood-Casts (see Fig. 52).—Blood-casts are coagula of red blood-cells which have formed within the tubules. The term is also applied to any of the above casts with red blood-cells attached. Some of the blood-cells are so pale that it is hard to recognize them.

Hæmoglobin.—Casts of hæmoglobin are seen in hæmoglobinuria, the hæmoglobin occurring in amorphous masses. Some seem like other casts impregnated with hæmoglobin.

Pus-casts (see Fig. 50) are formed in similar way as the blood-casts. The nuclei must be seen and their polymorphous nature certain, to be sure it is not an epithelial cast. This may be done by adding

acetic acid. Another point to differentiate from epithelial casts is the spherical shape of the pus-cells. These casts are rare, yet often other casts are seen with leucocytes attached which go under the same name.

CYLINDROIDS (see Fig. 53).—It is common and right to divide these into two groups. The first is of the so-called mucous threads, which are flat ribbons of mucus which no one would confuse with hyaline casts. Their length is considerable, several fields in fact; they vary in diameter, on the whole are narrow, and clearly show their ribbon-like nature. Such threads make up the nubecula. In addition to these and differing much from them in appearance are seen elements which look much like hyaline casts for the most of their

FIG. 51.—Hyaline casts of urine. × 400.

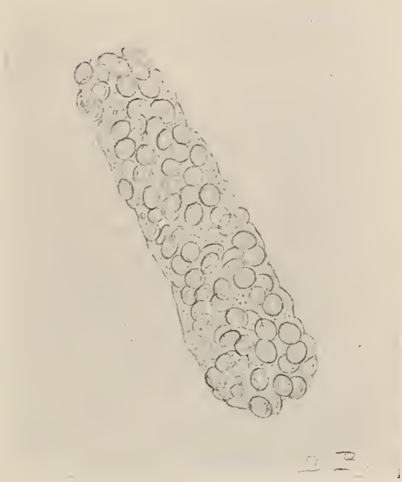


FIG. 52.—Blood-cast. × 400.

length, but at one end run off into a longer or shorter thread. Those particular on this point exclude from the list of casts anything which has one end at all tapering and thread-like. These casts appear to be circular on cross-section. They have not the fibrillar nature of mucous threads. They occur where casts would be expected, practically always with true casts, and have the same significance as they. Of one thing we are quite certain, that for the most of their length they are typical hyaline casts, and when, as may occur in the centrifuge, the thread-end is broken off, the other fragment could not be distinguished from a cast. The cylindroids may be covered by urates, and hence have the appearance of granular casts. Chemically these are like casts. The point is of considerable importance, for if mucous threads they certainly arise from the mucous surface, while if casts they should arise in the renal parenchyma. They were first described as of

some significance as casts. It is perhaps safest to observe the old rule and exclude all from the list of casts which have a definite tail at one end. The true mucous threads are insoluble in acetic acid. Their origin is the bladder chiefly. One seldom sees them in urine catheterized from the pelvis of the kidney.

COMBINED CASTS.—A cast may be waxy or hyaline at one end, granular at the other, or may have cellular elements attached. They take their name from the latter.

BACTERIAL CASTS.—Masses of bacteria in the shape of a cast occur in purulent infectious pyelonephritis and in pyæmic kidneys, but a cast also may become permeated by bacteria, either in the body or after voiding, in a remarkably short time.

URATE CASTS.—In uric acid infarcts of the kidney of the newborn masses of sodium urate may be found in the urine.

FIG. 53.—a, cylindroids, *i.e.*, bodies much resembling hyaline casts; b, mucous threads; c, a spiral structure of material resembling hyaline casts or mucous threads; d, a vegetable thread. × 400.

PSEUDO-CASTS of urates are commonly seen. It is not at all unusual for a urate sediment to assume this form. All casts in a concentrated urine may become incrusted with urates, and hence be of a dark homogeneous granular nature. True granular casts are not homogeneous, are coarser and more refractive than these pseudo-casts, which also have uneven edges and disappear on warming. Scratches in the glass are sometimes confusing (Fig. 54). In some cases of pyuria (cystitis, *e. g.*) the pseudo-casts are very perfect (Fig. 47).

The *length* and the *breadth* of casts vary. Their size may be from very small fragments to 1 mm. in length or longer. These very long casts are almost always hyaline, and are not mucous threads. Their diameter is narrow or broad. From

the size of the casts no conclusions can be drawn of their source, so much does the size of the tubules vary in various conditions. It was formerly supposed that the beautiful corkscrew forms so often seen came from the convoluted tubules, but this is improbable, since any corkscrew shape would certainly be effaced during their passage through the straight tubules. Some are spiral all the length, others only at one end. This, says Senator, merely shows them composed of a tough elastic material which has been squeezed through a narrow canal or through a narrow orifice. The end of the cast is seldom split or forked. Some casts are almost on the limit of vision, but vegetable fibres must be excluded.

The *origin* of epithelial casts, especially those with a lumen, is not disputed; also that of blood and pus casts, which are conglomerates of cells in tubules. The coarser granular casts quite certainly are formed from epithelial casts by a granular degeneration of the cells, and all transitions from the coarsely granular casts to the waxy may be found. This transition may also occur in the leucocyte casts, but is less true of the blood-casts. Such transitions are followed best by the study of sections of the kidney. The origin of the hyaline casts, however, has long been in dispute, some claiming that they were a coagulated exudate from the

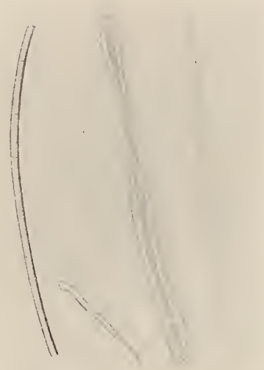


FIG. 54.—Pseudo-casts. From left to right, a linen thread, a vegetable spine, a cotton thread, and a scratch on the glass slide.

blood into the tubules, others a product of the secretion of the epithelial cells. Hyaline globules are present in these cells, the confluence of which in the tubules could easily explain casts; that is, the very slightly injured epithelial cell, still functioning, may by an abnormal secretion of coagulable material form these moulds of the tubules. This latter is the generally accepted idea. Also these epithelial cells could die, in part or wholly, undergo hyaline degeneration, and this substance be moulded into a cast. This also may be seen in sections. They are certainly not coagulated fibrin, since they arise where there is no suspicion of inflammation, that is, in a practically normal kidney, as in the albuminuria of the new-born; they do not give the Weigert's fibrin stain; where a coagulable albumin is present, as in the case of chyluria, these casts are absent; also there is no relation between albumin and casts in amount, and one may be present without the other. There is therefore no evidence for the albumin of the blood as their source. It is possible that a difference in origin explains the two forms of hyaline casts. Since casts which remain long in the tubules in cases where hyalines are the rule are waxy, the transition from hyaline to waxy must be considered.

The origin of hæmoglobin casts is interesting, since hæmoglobin is soluble in the urine. It may be, however, that they are hyaline or waxy casts impregnated with hæmoglobin.

The chemistry of the cast is little studied; probably none are fibrin. Some give an amyloid reaction when there is no such degeneration of the kidneys. Amyloid disease rarely has such casts. Some react reddish to gentian-violet, and brownish to Lugol's fluid, but a good Lugol reaction is rare. Their solubility in acetic acid is an important point in excluding mucus cylindroids.

Diagnostic Importance of Casts. It is claimed that casts may be present in normal urine. This is considered quite doubtful. They occur in any condition where albumin does or might occur, and it is agreed that any great number, even of hyaline cysts alone, means an irritated condition of the kidney, although a few may not. But although usually associated with albumin, either one may be present without the other. Osler has emphasized the point that in diagnosis it is not so much the casts, their number and variety, which are of prime importance as other urinary features (low specific gravity, etc.) and the physical signs of the patient.[154] Casts always mean, it is thought, some pathological condition of the renal epithelium, although this may be very slight, as a temporarily disturbed nutrition, but not necessarily nephritis. Their chief occurrence is in nephritis, but a similar urine picture is seen also in congestion of the kidney and in slight circulatory changes. In cases of albuminuria not due to nephritis casts may fail, and some (Bard) say when they cease in nephritis the inflammatory process has stopped. Their number is greatest in acute and chronic parenchymatous nephritis, fewest in contracted kidney, amyloid disease (see page 286), and in chronic passive congestion, While they may mean merely a temporarily disturbed nutrition of the cells, on the other hand they may mean their total destruction. The fatty, epithelial, or hyaline casts with fatty cells attached indicate a degenerative process. Casts with leucocytes attached may mean inflammation; blood-casts, hemorrhage, etc. In nephritis the casts and albumin, as a rule, run parallel. Exceptionally the casts may fail in a true nephritis, as in a case of jaundice or arteriosclerosis. Another very good illustration is the transitory albuminuria of pneumonia. With a failure of albumin the casts may persist. This is considered common in very contracted kidneys. In this connection it is to be remembered that casts will rapidly dissolve in the urine, and hence the presence of albuminuria without casts is not nearly so interesting as is the reverse condition. This disappearance of the casts may be easily demonstrated by comparing the urine after it has stood for some time, even though it remains clear, with that which is centrifugalized and the sediment examined while the urine is still warm. This disappearance of the casts has been attributed to the ferments present in the urine. Certain it is that if pepsin be present the casts

[154] New York Med. Jour., November 23, 1901.

could not remain there long. Trentlein says it is not pepsin but bacterial (colon bacillus) ferments.

"Showers" of casts are especially interesting. If a case of nephritis be followed daily, on certain days large numbers of casts will be found, and then in a day or so very few. Unless repeated examinations are made, a good idea of the number of casts cannot be gained.

The presence of casts without albumin is not rare if one centrifugalize well the urine. Such cases of pure "cylindruria" seem due to some evidently slight irritation of the kidneys, due to foods—asparagus, radishes; to coffee, mustard, and alcohol (Gläser found, after an even mild use of alcohol in some cases, casts and leucocytes, calcium oxalate and uric acid crystals, the effect lasting about thirty-six hours); certain drugs, especially well studied after salicylic acid, mercury, inunctions, Fowler's solution, and camphor; after tuberculin injections; in valvular heart disease and arteriosclerosis; in cancer; in the milder grades of certain diseases, erysipelas, tuberculosis of the lung, diseases of the nervous system; in jaundice especially; constipation and diseases causing constipation (confirmed by Wallerstein in animals); inflammatory intestinal diseases; acute infections, especially typhoid and scarlet fevers; even in a case of uræmic convulsions with recovery (Tonelli); and in amyloid disease sometimes. In the above cases the casts are hyaline and granular, even epithelial, and blood-casts, and yet no albumin. In some of these cases there is perhaps the possibility that the urine, had it been diluted, would have given the test. For the past two years we have paid this point particular attention and examined all in the hospital in which this condition was present, and we find that in many cases of transient albuminuria the cylindruria will outlast the albuminuria. This is particularly true in those cases of mild nephritis to be attributed to a parenchymatous lesion; that is, to an acute process. In cases of arteriosclerosis we find that the reverse is more common, and have even seen cases in which this point has been of diagnostic value. The occurrence of casts and "nucleo-albumin" is a very common occurrence (see page 211).

In a case of brain tumor recently in our wards the urine contained waxy and epithelial casts and many cylindroids, yet no albumin.

It is very interesting that Müller, in connection with the "physiological" albuminuria of bicycle racers, mentions one case with many casts but no albumin. Sometimes the cylindruria is very transitory, lasting but thirty-six hours.

Pure cylindruria occurs in some cases of chronic nephritis and of amyloid kidney. Steward tries to separate a group of severe nephritis

cases which presents this feature, but the anatomical evidence for such a group is still lacking.

In cylindruria not due to nephritis the casts are few, and those found are hyaline, as a rule.

The casts of chronic passive congestion are scanty if any, and nearly all are hyaline. Yet all gradations occur between this condition and true nephritis.

It was formerly supposed that the epithelial and the hyaline casts meant an acute process, the granular and the waxy a chronic process; but in any form of nephritis all kinds of casts may occur; in amyloid disease even there is in the urine nothing characteristic. Sahli suggests that the granular and the waxy casts become such from lying a long time in the tubules, which occurs in acute nephritis or acute exacerbations of a chronic case, in which the secretion of urine is much suppressed. We have noticed this point also in other oligurias following, for instance, decapsulation of the kidneys. For the first few days, as the urine begins to increase in amount, a very large number of waxy casts were found.

Following renal disturbance of almost any kind casts may appear. Brown [155] has reported some interesting cases in which operations upon the kidney, nephropexy or exploratory nephrotomy, the urine previously being normal, were, the first day after the operation, followed by an output of casts in enormous numbers. These were hyaline, granular, blood, and epithelial. The field may be really crowded with them. Considerable albumin may be present. They diminish rapidly in amount, and in a few days (from two to six) have entirely disappeared. During this time there have been no symptoms of nephritis, no œdema, and the amount of urine has been normal. The most striking urinary feature was the disproportion between the amount of albumin and the number of casts, this being greatly in favor of the latter. There were no later symptoms.

Epithelial and leucocyte casts are not nearly so rare as is imagined, and may occur in even non-inflammatory transitory cylindrurias.

Casts are considered important as a prodromal symptom of diabetic coma (Külz). Before the coma begins casts appear in immense numbers, and even may form a macroscopic sediment. These casts are of characteristic appearance,—short, broad, of delicate outline, pale, the most of them granular and hyaline, and with few other formed elements.[156]

Staining Casts.—This is unsatisfactory, because the stain precipitates in the urine, or the albumin of the urine may itself take the stain. The specimens cannot

[155] Johns Hopkins Hosp. Bull., May, 1900.

[156] See Domansky and Reimann, Zeitschr. f. Heilk., 1901, and Herrick, Am. Jour. Med. Sci., vol. cxx. 1900.

be dried for this reason. The casts should be washed by one or two sedimentations with 0.6 per cent. sodium chloride solution to rid of all soluble matter and albumin. In the next centrifugalization 1 per cent. methylene blue may be added. To hasten centrifugalization a little alcohol should be added, not much, nor allowed to remain in contact with the sediment for a long time, else a coagulum will result.

To preserve the casts and also to stain them they should be washed in the above normal salt solution, and lastly in a 1 per cent. osmic acid solution or in 1 to 10 per cent. formalin, or in a 5 per cent. $HgCl_2$ solution for five minutes. In the latter case they are then washed with water and preserved in from 2 to 10 per cent. (or 1 to 2 per cent.) formalin. If no red blood-cells are present, the mercuric chloride may be omitted. This salt disturbs microchemical tests. In case formalin is used the casts should be well washed once or the spherical crystalline masses of diformaldehydurea will form. Gumprecht adds that it is not really necessary to wash the casts if they are well centrifugalized and the supernatant fluid completely decanted. A good staining method for fat and cell nuclei was described by Cohn.[157] The specimen, well washed by centrifugalization in normal salt solution, is air-dried on the cover-glass and hardened by immersing the glass in 10 per cent. formalin for ten minutes. It is then washed rapidly but gently with H_2O and then immersed for ten minutes in a concentrated Sudan III. solution in 70 per cent. alcohol. It is then washed in 70 per cent. alcohol for one to two minutes and then stained briefly in the hæmatoxylin (Ehrlich's). The specimens are mounted in glycerin.

Kozlowoski[158] recommended Farrant's mounting fluid, consisting of equal parts of water, glycerin, and saturated aqueous solution of arsenic acid (saturated by weeks of standing); to this gum arabic one-half volume is added, and the whole allowed to stand (about three weeks) till all is dissolved. It is then filtered if necessary.

In the centrifuge tube are mixed 1 cm. of 1 per cent. eosin or methyl violet with the urine, then centrifugalized and washed by centrifugalization till all the urine is removed. One drop of sediment is then mounted on the slide with one drop of the above fixing fluid.

Bohland advises to wash with salt solution; then Müller's fluid is added, and they kept in this for two weeks, changing the fluid two to three times. The Müller's fluid is then decanted and the sediment washed in absolute alcohol until this is colorless.

Testicular Casts.—In "spermatorrhœa" casts have been described which "can hardly be distinguished from renal casts except that the urine is otherwise normal. They are all in the first glass of the two-glass test, and the presence of spermatozoa will indicate their origin. They are supposed to arise in the testicle." We have inquired of those with a very wide experience in the examination of prostatic secretions, and they say they have never seen such structures. *Spermatozoa* may often be found, active at first, and with all of the elements of unripe semen. They soon go to pieces. Such occur not only after coitus and pollution, but after epileptic and other convulsive seizures.

Tripperfäden.—These occur in a late stage of acute gonorrhœa and in chronic gleet when the secretion becomes very mucous and collects in the longitudinal furrows of the mucosa. They may be from a few millimetres to one centimetre long and yellow or white in color,

[157] Zeitschr. f. klin. Med., 1899, Bd. 38.
[158] Virchow's Arch., 1902, vol. clxxix. p. 161.

consisting of a mucous ground substance in which are embedded pus and epithelial cells.

Tissue fragments, portions of carcinoma, have been found; also masses of caseous matter in cases of tuberculous ulceration. The elastic tissue may be demonstrated, and in the case of cancer the spindle cells, which may enclose hæmatoidin crystals and red blood-corpuscles. To demonstrate elastic tissue the urine should be centrifugalized, acid added to dissolve the phosphates, the supernatant fluid decanted, and the sediment then warmed with an equal amount of 10 per cent. KOH. This destroys all but the elastic tissue. It is then sedimented again and examined microscopically.

In papillomatous cancers and growths of the bladder fragments large enough to be cut in sections may be found. They have been found in the urine especially of cases of carcinomata of the bladder, very rarely of the kidney. From some of these a diagnosis could be made. Fragments of sarcomata in the urine are almost unknown, but structureless masses have been found, as by Rothschild,[159] from a giant-cell sarcoma of the kidney. This large mass was 5.2 cm. long and 0.5 cm. wide, structureless, glassy, transparent, and quite firm.

Other gross masses are mucous casts (see page 211), and fibrin masses in chyluria, hæmaturia, and from inflammatory conditions, especially tuberculosis.

Pus-Cells.—These occur, a few perhaps, in normal urine, but many in any inflammation of the urinary passages, of the kidney, or in case an abscess ruptures into the urinary tract. Their numbers vary enormously. As a rule, if from the cortex of the kidney, they are few in number, if from the passages, many.

Hottinger found in a case of cystitis 150,000 leucocytes per cubic centimetre, that is, a daily output of about one one-hundredth the number of leucocytes normally at one time in the circulating blood.

Their origin is better indicated by other constituents, as, for instance the character of the epithelial cells or the presence of casts. In case a large amount of pus appears suddenly, the probable source is ruptured abscess. The pus-cells in gonorrhœa may be mixed with mucus, form threads, the so-called Tripperfäden which probably are formed in the folds of the mucosa and are washed out by the stream of urine. These Tripperfäden will settle to the bottom of the glass. They should be searched for only in fresh specimens by agitating the urine, when they arise as long threads, since if allowed to settle long they will coalesce. They have a considerable diagnostic value.

In all cases of women pus from the vagina should be excluded.

In alkaline urine the pus-cells will swell and the mass become slimy and gelatinous. The pus sediment is, as a rule, slimy, since it

[159] Deutsches med. Wochenschr., 1901, No. 50.

contains so much mucus. Albumin is always present. Microscopically in acid urine the cells may be cloudy and shrunken, and acetic acid be necessary in order that the nucleus may be seen at all. In an alkaline urine they will swell and become glassy, but even here it is not easy to see the nucleus. In a weakly alkaline, amphoteric, or faintly acid urine they may be well preserved for a long time, and even show active amœboid motion. Their diameter is from 7 to 12 microns. Their nucleus is small, usually polymorphous, never vesicular. This will exclude renal epithelium, which it is often hard to do except in stained specimens. (Senator considered that many of the pus-cells in Bright's disease were mononuclears.)

We have had opportunity recently to examine two cases in which the leucocytes were so drawn out that they resembled spindle epithelial cells. This may have been due to long centrifugalization in one case. This was true of practically all the leucocytes and on two preparations.

It is often important to decide whether there is more albumin than the pus serum would explain; that is, if true albuminuria is also present. In case casts and renal epithelium are found, that will probably be the case.

Posner's Method.—The albumin is estimated carefully. The urine is well shaken (a twenty-four hours' specimen) and the leucocytes counted with the ordinary blood-counter. For each 100,000 leucocytes per 2 cc. of urine, one may expect 0.1 per cent. albumin (Goldberg, 2 p. M.). The urine must be diluted with 1 to 3 per cent. NaCl solution if over 3000 per cubic millimetre are present.

The same author has given an easier method which is of some value. The urine is poured into a flat-bottomed beaker which is placed over a sheet of ordinary printed paper. The well-shaken urine is then poured in until the type cannot be read. Normally one can read through a layer of urine 8 cm. deep; if the type cannot be read when the layer is from 0.5 to 1 cm. it indicates 40,000 leucocytes per cubic centimetre; if 6 cm., 1000 leucocytes per cubic centimetre. The benefit of treatment may be followed by this method. A former idea, that the filtrate of a urine without true albuminuria is albumin-free, is hardly true, although the albumin in the filtrate is of very small amount.

Donné's Pus Test.—The supernatant fluid is poured off, and to the sediment a small piece or strong solution of KOH or NaOH is added. If the sediment is pus it will be transformed to a viscid gelatinous mass which sticks to the glass.

The pus-cells will take a mahogany-brown color with Lugol's.

Red Blood-Corpuscles apart from True Hæmaturia.—These are present in the urine in acute inflammations of the kidney, tumors of the urinary passages or kidney, in cases of trauma, stone, chronic pas-

sive congestion, the hemorrhagic diathesis, and in many trivial conditions in which they would not be expected. Some of the cells are intact or they may be changed by the urine. It is particularly common to see merely shadows or rings of the swollen decolorized cells.

In concentrated urines they are crenated; in dilute, swollen or laky; in acid urines, intact; in alkaline, destroyed, forming masses of yellowish-brown granules.

It is important to decide if these cells come from the cortex of the kidney or not. A cortical origin may be assumed if many red blood-cells are sticking to casts or if true blood-casts are present. An amount of blood sufficient for clot formation usually has its source below the cortex, but sometimes in nephritis enough blood may escape from the cortex to form large clots, the shape of which will sometimes indicate the source, it being a cast of the pelvis or of the ureter.

Gumprecht claimed that if many of the reds were found fragmented, that is, present as clumps of granules, the source in the cortex is to be assumed, since here alone the urea solution is strong enough to fragment the reds (8 per cent.). Goldberg thinks that these cells can become fragmented in a distended infected bladder.

Renal and Bladder Stones.—By renal stones are meant all from the pelvis of the kidney and the ureter. They vary in size from a grain of sand to those which fill the whole pelvis of the kidney. These large ones with the branches may be hollow, furnishing thus a passage for the urine (in one case it weighed 1088 grains). The bladder concretions are single or multiple, and vary greatly in size.

Uric Acid Concretions.—These are very common, the most common renal stone. The size of those found in the bladder varies from that of a pea to that of a goose-egg. They are always colored, most commonly a grayish yellow, yellowish-brown, or a pale reddish-brown. The surface is sometimes smooth and polished, sometimes rough and nodular. They are very hard, fracture easily, and on cross-section they show a concentric arrangement and crystalline structure, the layers of which may be separated being of different colors. These layers may be alternately of uric acid and some other salt, as, for instance, CaOx. They burn without residue if pure, they give the murexid test, on the addition of NaOH they liberate but little ammonia, are soluble in alkali, and this solution plus acetic acid gives crystals for the murexid test.

Ammonium urate stones are primary in the new-born, and occur rarely in adults. As secondary deposits they occur more commonly. These stones are almost as soft as wax, when dry are clayey and easily powdered. They give the murexid test and with NaOH liberate much ammonia.

Calcium oxalate stones are, next to uric acid stones, the most common,

and yet seem the most numerous, since they cause severest symptoms. They are either smooth and small or very large with a rough nodular or ragged surface, of a dark gray to blackish color. They cause hemorrhage easily, and hence are often stained dark brown with blood-pigment. They are the hardest and heaviest of stones. They are soluble in HCl without gas formation, but not in acetic acid. After moderate heating, however, the powder is soluble in acetic acid with gas evolution. After strongly heating, the powder reacts alkaline because of the $Ca(OH)_2$ formed. They contain almost always some uric acid, xanthin, or calcium carbonate, and hence have a concentric layer arrangement.

Phosphate Stones.—As renal stones they are rare and small, yet often occur as ingredients of other stones. They usually consist of a mixture of normal phosphates of the alkaline earths and triple phosphate. In the bladder they may be very large. They occur especially as secondary concretions, and contain ammonium urate, CaOx, and carbonates. They often form around a foreign body. Their color varies,—sometimes white or pale yellow, or purplish. They are soft, light in weight, the surface always rough. Concretions of triple phosphate alone are rare. They are small with a granular surface, upon which are often red crystals. Stones of the ACID CALCIUM PHOSPHATE are rare. They are white and of a beautiful crystalline structure. These phosphate stones do not burn, the powder is soluble in acetic acid without gas formation, and the solution gives the reaction of phosphoric acid and of alkaline earths. They usually contain a great many organisms. The TRIPLE PHOSPHATE STONES liberate much ammonia on the addition of NaOH.

Calcium carbonate stones are rare in man, are chalky white, soluble in acid with gas formation.

Cystin stones are rare. Their size varies, and may reach that of a hen's egg; as renal concretions they are seldom larger than a small pea. The life of some of the cases of cystinuria is wretched because of the rapid formation of stones large enough for operation. They are light in weight, smooth, soft and waxy in consistency, hence may usually be crushed. They have a smooth or ragged surface, are white or pale yellow, and crystalline on cross-section. They are rather wax-like, burn readily and perfectly on a platinum-foil with a bluish flame, are soluble in ammonia, recrystallized by acetic acid, and give the other cystin reactions.

Xanthin stones are very rare and occur especially in children. This is also a primary formation. They vary in size from a pea to that of a hen's egg. They are pale white, yellowish-brown, rather hard, amorphous on cross-section, and on rubbing appear like wax. They burn without residue on the platinum-foil, and give the xanthin test.

Fatty concretions have only a few times been observed. They contain free fatty acid, neutral fat, and much cholesterin. In some cases these have been found to be of the fat used in passing bougies.

Indigo.—Three such stones are on record, and yet this may be the nucleus of various other stones. They have a blue or bluish-gray surface.

Albumin.—One such calculus is on record.

WHEN HEATED ON THE PLATINUM-FOIL THE POWDER

(HOFMEISTER'S TABLE.)

<table>
<tr><td colspan="4">Does not burn</td><td colspan="6">Burns</td></tr>
<tr><td colspan="4">The powder + HCl</td><td colspan="3">With flame</td><td colspan="3">Without flame</td></tr>
<tr><td colspan="3">Does not effervesce</td><td rowspan="4">Effervesces.</td><td rowspan="4">The flame is yellow, continuous, odor of burning feathers. Insoluble in alcohol and ether; soluble in hot KOH, and reprecipitated white by acetic acid, with H_2S formation.</td><td rowspan="4">A yellow, clear continuous flame, odor of resin or shellac; the powder soluble in alcohol and ether.</td><td rowspan="4">Flame pale blue, burns for a short time with a characteristic sharp odor. The powder is soluble in NH_4OH and on evaporating hexagons are precipitated.</td><td rowspan="4">Does not give the murexid test. The powder is soluble in HNO_3 without gas effervescence, and this dried residue becomes orange when KOH added, then red on warming.</td><td colspan="2">The powder gives the murexid test.</td></tr>
<tr><td colspan="3">The powder moderately burned + HCl</td><td colspan="2" rowspan="2">The native powder on the addition of a little KOH in the cold</td></tr>
<tr><td colspan="2">Does not effervesce
The native powder moistened with KOH</td><td rowspan="2">Effervesces.</td></tr>
<tr><td>Gives off much NH_3. The powder is soluble in acetic and HCl, and a crystalline precipitate formed with NH_4OH.</td><td>Gives off little or no NH_3. The powder is soluble in HCl and acetic acid—an amorphous precipitate falls with NH_4OH.</td><td>Gives off much NH_3.</td><td>Gives off little or no NH_3.</td></tr>
<tr><td>Triple phosphate</td><td>Neutral Ca or Mg phosphate</td><td>CaOx</td><td>$CaCO_3$</td><td>Fibrin</td><td>Fat</td><td>Cystin</td><td>Xanthin</td><td>Ammonium urate</td><td>Uric acid</td></tr>
</table>

Bacteria.—The growth of bacteria in urine on standing is exceedingly rapid, and the rapid changes which they make in its chemical composition necessitates the use of substances like chloroform, camphor, thymol, etc., to preserve it. These bacteria are usually contaminations, but in cases of infections of the kidneys, urinary tract, and in cases of the " excretion" of bacteria, perhaps without local renal lesion, their examination is of particular importance. Cloudiness due to them will not clear on sedimentation nor filtration. In old urines these organisms form a scum on the surface, and in cases of bacilluria the fresh urine will sometimes shimmer, when shaken, like a bouillon culture.

That the bacterial examination may be valuable it is necessary that the specimen be catheterized with the greatest care. In certain diseases of the urinary passages, especially the bladder, saprophytes may grow in great numbers, causing an intravesical ammoniacal decomposition of the urine. The organism to which this is usually ascribed is Micrococcus ureæ, soon present in every urine, but since normally not when fresh a special ferment is assumed. Of the pathogenic organisms found in infectious nephritis, cystitis, etc., are the colon bacillus especially, streptococci, and staphylococci, and further the typhoid, which may be present in enormous numbers and with hardly any pus in perhaps one-third of the cases of typhoid fever. So numerous may these be (even five hundred millions per centimetre, Gwyn) that on shaking the fresh urine one obtains the same shimmer seen in the bouillon culture. Actinomycoses have also been found. In one case of cancer of the stomach the lactic acid bacillus (of Boas) was also present in a cancer nodule of the kidney.

In cases of suspected tuberculosis of the kidney or other accessory organs it is often important to demonstrate the TUBERCLE BACILLUS. This is often difficult, and various methods have been proposed. The great trouble is to wash the specimen clear of urea which because of its hydroscopic properties renders a good smear almost impossible. This may be done by washing the sediment several times on the centrifuge with water, in which case, however, a little egg albumin must finally be added to stick the bacilli to the slide. (For staining methods, see page 50.)

If much sediment is present the search is easier, since fragments of tissue contain the bacilli hence a good smear is possible.

In centrifugalizing it is well to add two volumes of alcohol to the urine, since this, because of its lighter specific gravity, renders centrifugalization possible. Gregersen advised sedimentation in preference to the centrifugalization, since in the latter case the organisms stick to the glass. Animal inoculations are often necessary.

The SMEGMA BACILLUS is generally much feared, and very many stains (even twenty-one) have been proposed to exclude it. In gen-

eral, it may be said that if care be taken to clean the external genital organs, the folds of the prepuce or clitoris and the external orifice of the urethra, there is no danger. The only hope to exclude this organism is by not getting it, rather than by any stain or morphological test, for while many forms of the smegma bacillus may be thus recognized, some cannot. The smegma bacillus varies much in morphology and staining characteristics; no one feature is constant, and some forms are exactly like the tubercle bacillus in all these points. It lives anywhere on the surface of the body that the secretions of the skin accumulate. One avoids it by external cleanliness, using special nozzles for irrigation, and then calls any acid-alcohol-fast organism in the urine a tubercle bacillus; yet even then the court of last appeal is animal inoculation.

Since the tubercle bacilli have been found in cases of acute miliary tuberculosis without especial renal involvement, and some think may be excreted by the urine in tuberculosis of other organs than the kidneys, their presence without any other local sign or urinary feature is hardly enough to justify the diagnosis of renal tuberculosis.

Churchman gives a full discussion to this question (Am. Jour. Med. Sc., July, 1905).

The YEAST CELLS occur especially in diabetes, since they require a sugar medium. In this disease they may consume the sugar before the urine is voided and give rise to "pneumaturia." They also occur in alkaline urine and may form a sediment. To cultivate them the urine should be kept acid with acetic acid.

MOULDS may perhaps sometimes occur in the urine when voided, but the spores accumulate on standing. In a recent case of pyelitis, the pelvis of the kidney had been repeatedly irrigated through ureteral catheterization, on one occasion the urine which escaped was very bloody, and in it were found mycelial masses of some organism which would not grow on media. After washing the pelvis out well no more were found. They may have been introduced at a previous catheterization.

SARCINÆ smaller than those found in the gastric cases may also be found.

Among the **Animal Parasites** which may be demonstrated either partly or entire in the urine are the hooklets, daughter cysts (even several hundred in a case), and fragments of membrane of ECHINOCOCCUS CYSTS. There are no urinary symptoms of hydatid disease of the kidney unless there be catarrhal pyelitis or the cyst empties into the urinary tract. If the latter be the case the urine will appear watery, soapy, or bloody. Embryos of filaria are found in tropical hæmatochyluria. (See page 608.)

FLAGELLATES belonging to the cercomonas or the trichomonas class occur. Concerning these there is a dispute as to their origin, whether present before voiding or a later contamination of the urine.

Eustrongylus Gigas.—A few cases are reported, but in many they were mistaken diagnoses. In one case of chyluria these eggs were found.[160]

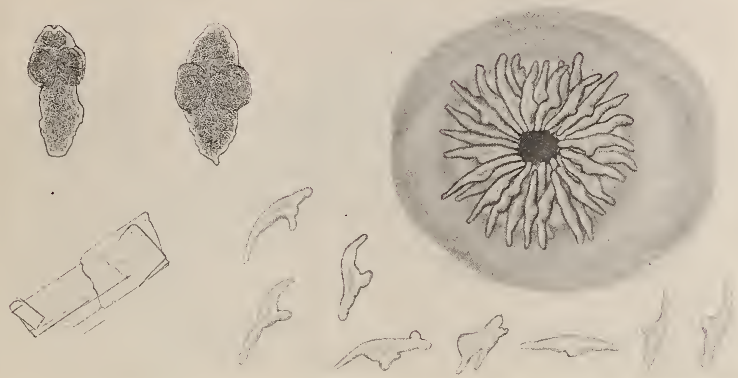


Fig. 55.—Sediment from echinococcus cyst. Above and to the left are two degenerated scolices (× about 60); to the right is the head of a scolex (× 400); below are hooklets of unusual shapes and a small mass of cholesterin crystals. × 400.

Schistosomum Hæmatobium (Bilharz). Distoma Hæmatobium.—This worm, so common in Africa, especially Egypt and the Transvaal, has been found but six times in this country[161] and twice in Porto Rico (Martinez). The adult male (see Fig. 57) is 12 to 14 mm. long, flat, but so folded that it forms a gynecophoric canal which receives the female, which is 20 mm. long and filiform.

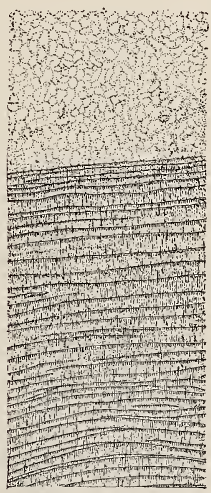


Fig. 56.—A small fragment of echinococcus cryst-wall on cross fracture, showing transverse striation and pectination. × 50.

The adults live in the portal system and the mucosa of the urinary tract and rectum, also in the pelvis of the kidney. The results of its presence are hemorrhages, "the Egyptian hæmaturia," either profuse or but a few drops at the end of voidings, pyelitis, even atrophy of the kidney. The eggs (Fig. 92) are voided and may become the nucleus of a stone. The symptoms are hæmaturia with these eggs. They are large,—0.16 by 0.6 mm. in size,—with a transparent shell, and contain a ciliated embryo.

Nematode worms other than filaria are sometimes found in the

[160] Stuertz, Deutsches Arch. f. klin. Med., 1903, vol. lxxviii. p. 586.

[161] See O'Neil, Boston Med. and Surg. Jour., October 27, 1904, vol. cli. p. 453.

urine, some of which may be *Anguillula aceti*, or "vinegar eel." Stiles reports one case of infection of the bladder with this worm. Other cases may be due to contamination from the bottle in which the urine is collected.[162] These worms resemble closely Strongyloides in-

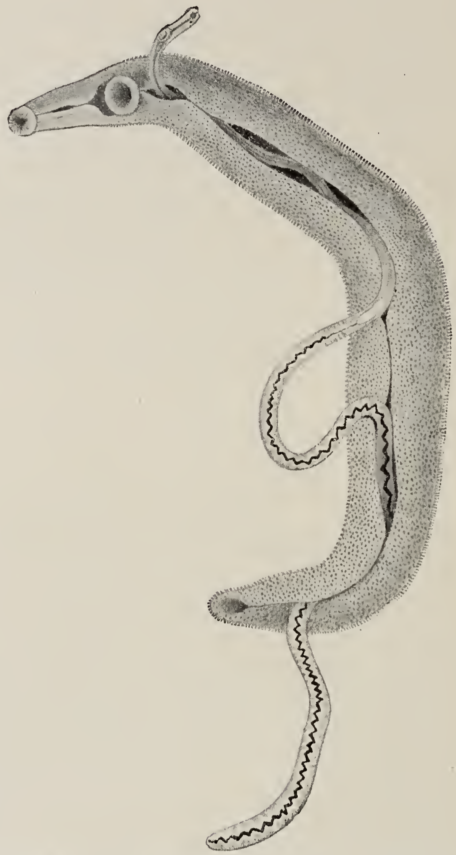


FIG. 57.—Schistosomum hæmatobium, adult worms. (Copied from Braun.)

testinalis, except that these worms (A. aceti) are slightly longer. (Males, 1.2 mm. long, 0.033 mm. wide; females, 1.9 mm. long, 0.06 mm. wide; embryos, 0.25 to 0.3 mm. by 0.015 mm.)

The student should always be able to recognize the various plant contaminations which occur in water and hence in vessels rinsed out with this water; that is, he should be able to say that they are plants and of no significance. A few of the most common we give in Fig. 58.

Prostatic Fluid.—This fluid is best obtained by "milking" the prostate through the rectum. The urethra is first well washed, then the

[162] Billings and Miller, American Medicine, May 31, 1902.

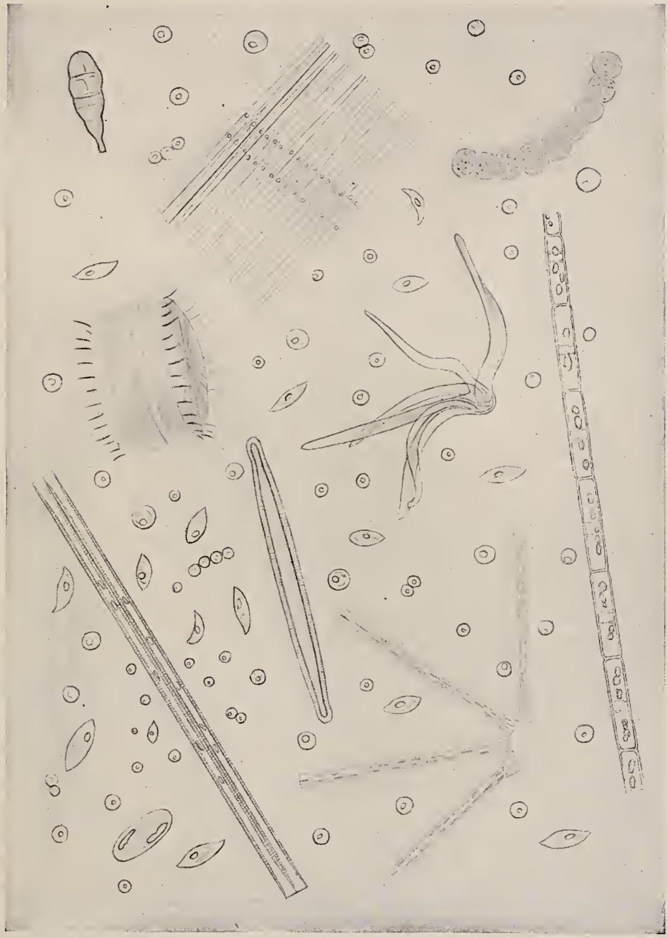


FIG. 58.—Protophytes and other low forms of life often found in tap water. × 400.

fluid expressed and collected. The amount varies greatly, from none to even 5 cc. of normal fluid at one milking. It is of a grayish-white, yellow, or greenish color, with a milky turbidity due to lecithin globules, and of a characteristic odor. It is slightly viscid, tenacious, of light specific gravity since the solids are but 1 to 2 per cent., faintly alkaline as a rule, although it is acid to some reagents; its reaction varies very much, and is as yet a very uncertain quality.

One examines first fresh for motile spermatozoa, then adds a drop of acetic acid to bring out the cells more clearly, and examines for pus-cells. (Fig. 59, b.)

Microscopically, the most striking objects are the great numbers of *lecithin globules* (Fig. 59, a), which give it its milky appearance. These vary in size from those minute to others even half the size of a

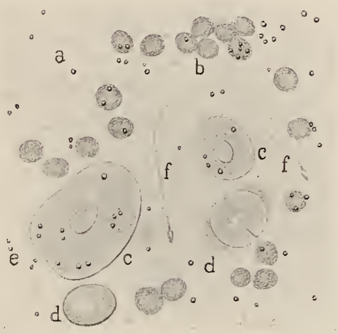


Fig. 59.—Prostatic fluid. (× 400.) a, lecithin globules; b, pus-cells; c, epithelial cells; d. corpora amylacea; e, free granules from epithelial cells; f, spermatozoa.

red blood-cell. They are not very refractive, and can be easily distinguished from fat. Their only significance is that an increase is a good sign in cases of chronic prostatitis. *Corpora amylacea* (Fig. 59, d; 60, c) are often found, especially in advanced life, in which case they also appear in the urine. They are laminated, with a finely granular centre, often a nucleus. Of their composition nothing is known except that they stain blue with iodine; they have no significance. *Epithelial cells* of various kinds are present. Some are large, polygonal, single or in groups, and of very varying size (see Figs. 59, c, 60, a). Other cells, the so-called granular cells (Fig. 60, e), also of varying size, are simple masses of granules resembling fat, some of which resemble colostrum corpuscles. These break down, hence the refractive globules seen free in the fluid (Fig. 59, e). Some granules resemble myelin (Fig. 60, d). Columnar epithelial cells are sometimes present. In addition to these are large clear cells of very varying size, with or without nucleus (see Fig. 60, b), which are supposed also to be derived from the seminal vesicles. There are normally no pus-cells, nor any red blood-corpuscles.

Spermatozoa (Fig. 59, f) are usually present. (For a description of these, see University of Pennsylvania Med. Bull., No. 3, 1902.)

These should be examined fresh, to make sure of their motility; to study them more carefully smears are made, dried in the air, heated to 120°, and cooled slowly. The fluid should first be diluted with water even of twenty volumes in case much proteid be present. They are best stained in iron hæmatoxylin. The specimen fixed by heat is placed in 2 per cent. iron alum solution for from two to four hours, washed in water, then in 1 per cent. hæmatoxylin for twelve hours. They are decolorized with 1 per cent. iron alum carefully, and counterstained with saturated aqueous solution of eosin from one to three minutes, then dried and mounted. Many of them are abnormal in shape, some with two heads and with even three tails. These monsters seem never to move.

One seldom tries to determine more than their presence and motility; if motile, one is confident that they are functionally normal; if absent or non-motile, no conclusions are justified.

In acute or chronic prostatitis many leucocytes are present, and lecithin is diminished.

Spermin crystals resemble somewhat the Charcot-Leyden crystals, being colorless, transparent needles or whetstones, but are often imperfectly crystallized. To demonstrate these the semen is allowed to stand. To the prostatic fluid, however, must be added one drop of 1 per cent. ammonium phosphate and the specimen allowed to dry under a cover-glass for two hours.

The PROSTATIC CASTS which are said by some to resemble the urine casts markedly must certainly be rare, since we have asked those who have examined many hundreds of specimens, and they have never seen one.

TRIPPERFÄDEN.—These threads, which are seen grossly in the urine in cases of chronic gonorrhœa, are present as narrow delicate transparent mucous flocculi, which microscopically contain mucus and a few epithelial and still fewer pus-cells; this form is present in very chronic cases; or shorter, firmer bands, in which the cellular elements, especially the pus-cells, predominate. They settle at once to the bottom of the glass, but are evident on agitating the urine, upon which the threads rise from the bottom. In an old urine they may be difficult to demonstrate, since they have coalesced.

Also short coma-like flocculi are seen which arise from the excretory ducts of various glands and follicles, and mean an intense involvement of the urethral glands. Those in the second glass are from the glands in the prostate, and are signs of chronic prostatitis. They consist of superimposed layers of cylindrical epithelium.

PROSTATIC PLUGS are sometimes found which are large cylindrical masses of mucus. These are found in mild inflammations of the prostatic ducts. Other mucous masses are found full of spermatozoa (Fig. 61).

FIG. 60.—Prostatic fluid. a, epithelial cells; b, clear epithelial cells, from seminal vesicles(?); c, corpus amylaceum; d, "granular cells" with droplets resembling myelin; e, "granular cells" with fat droplets.

DISEASES OF THE KIDNEYS

Albuminuria.—In order to get a general idea of the occurrence of albuminuria, the conditions in which it occurred most commonly, and, if possible, obtain some clue for further investigation in this important subject, we have abstracted the histories of 3631 hospital cases, taking them in order of admission to the hospital without reference to their diagnosis. Only such histories were abstracted the urine examinations in which appeared to us perfectly satisfactory.

It very soon became evident that the age line, that is, the occurrence of albumin in the various decades, is to be first determined. So important is this that the effect of any given agent or disease upon

FIG. 61.—Mass of mucus filled with spermatozoa from urine catheterized at death. × 400.

the kidneys can only be rightly determined in case the age be taken into consideration.

We have divided the cases into three groups,—those in which the urine was throughout the stay in the hospital albumin-free; those in which the albumin was present for a time but disappeared while under examination; and those in which albumin was present at each examination. As the age epochs we have chosen from one to fifteen, sixteen to twenty-five, twenty-six to thirty-five, thirty-six to forty-five, and so on through the epochs. The reason for choosing these figures is that the ages of fifteen and twenty-five are more truly transition points in a person's life than ten and twenty. Not only should the urine be studied by decades, but also the sexes should be studied separately for certain decades at least. On the whole, the sex has much less influence than one would expect. These figures will be published in full later. The neurasthenics may be taken as representing a group of normal men, for this diagnosis represents exclusion of other conditions. Of the men, the percentages with albumin-free urine were: one to fifteen years, 100 per cent.; sixteen to twenty-five, 87 per cent.; twenty-six to

thirty-five, 99 per cent.; thirty-six to forty-five, 90 per cent.; forty-six to fifty-five, 84 per cent.; fifty-six to sixty-five, 70 per cent.; sixty-six and over, 66 per cent. The drop at the period of adolescence is very interesting (see page 222).

Of the FEVERS, typhoid after the twenty-fifth year is accompanied by a transitory albuminuria (febrile) in 30 per cent. of the cases, and a persistent albuminuria in about 30 per cent. One would expect this, since the fever is so continued and bacilluria is common (about one-third of all cases). Yet as a disease in the past history, typhoid fever strangely enough seems to have the least effect on the kidneys, notwithstanding that it has a deleterious influence on the peripheral blood-vessels.

Malaria of the tertian and quartan types has little effect on the kidney, æstivo-autumnal much. Pneumonia has the highest percentage of transitory albuminuria of all the fevers we studied (but about 25 per cent. of the cases are albumin-free), but almost no permanent effect. Pulmonary tuberculosis and acute articular rheumatism cause little febrile albuminuria. Of the AFEBRILE DISEASES, the neurasthenics are the best off; the arteriosclerotics the worst. In fact, this disease, arteriosclerosis, seems the one dominating element among the causes of albuminuria.

The relation between the anatomical lesions and urinary findings was studied.

Cases with marked CLOUDY SWELLING at autopsy, but no other lesion, as a rule have had an albuminuria, usually slight, extending for two or three weeks before death, but in a few cases none even the days before death. Casts are also present with albumin, usually hyaline, but also waxy, epithelial, and blood.

FATTY KIDNEYS (no other microscopical changes) are seen in various diseases, and have been divided into those with fatty infiltration and fatty degeneration; the former occurring in diabetes mellitus, pregnancy, etc., the latter in various infectious diseases, cachexias, anæmias, and following various poisons.

The amount of urine is usually normal, although in some severe cases, decreased; albumin is present in various amounts, a trace or much, and a relatively large number of casts, hyaline, granular, fatty, and epithelial; with few or many red corpuscles.

All of our cases examined had excreted albuminous urine before death, but in no case exceeding two weeks. Hyaline and granular casts were present.

The urine of kidneys with CHRONIC PASSIVE CONGESTION is at first scanty, dark in color, very acid, the specific gravity between 1025 and 1030. The urate sediment is often abundant. Urobilin and uro-

erythrin are increased and sometimes bilirubin is present. Sooner or later albumin appears in traces, later in good amount, even 0.1 per cent., and in one of our cases 0.6 per cent. Casts are present, chiefly hyaline, rarely granular. Yet here also on some days casts may be present in great numbers, hyaline, waxy, epithelial, and fatty. A very few leucocytes may be found and still fewer red cells. The points of importance in this urine are the small amount of albumin, the large urate sediment, the absence of renal epithelium, the scarcity of granular casts and leucocytes. The diagnosis of nephritis had been made in over half the cases.

Acute Nephritis.—Acute nephritis has been divided into several groups, chiefly from the stand-point of pathology, but for the clinician a division is very difficult. Senator separates the tubular nephritis or acute parenchymatous from the acute diffuse. In the *acute parenchymatous* the tubules especially are involved, and the glomeruli little so, or not at all. The clinical symptoms are slight if any. The urine shows only a slight febrile albuminuria, a diminished amount of urine of rather high specific gravity, and few or no casts. From this form are all gradations to the acute diffuse nephritis. The urine contains often a heavy sediment, chiefly of renal epithelium, and hence the name "nephritis desquamativa." The epithelial cells may be single or in casts. Hyaline casts, few or many, are present. Crystals of uric acid and calcium oxalate are often present, red blood-corpuscles and hæmoglobin in granular casts or masses. The leucocytes are usually few in number. The albumin is nearly always slight in amount, in remarkable contrast to the amount of sediment, and some claim that nearly all of it is nucleo-albumin from the cells.

In the acute diffuse nephritis, a good illustration of which is that following scarlet fever, the clinical symptoms are much more severe. The urine is diminished in amount, there may, indeed, be anuria for the first twenty-four hours. From 50 to 100 cc. for the first day or so is not uncommon, but later from 200 to 500; specific gravity high, even 1030. Toward death there may be a diminished or an increased amount voided. The specific gravity was normal as a rule, 1015 to 1017, but in some cases high, from 1023 to 1025 (when the urine was from 300 to 600 cc.). It is usually of a dark color and cloudy. In very mild cases, however, it may appear normal. Blood is nearly always present. When slight in amount it imparts to the urine a slight smoky tinge, which may be recognized grossly. When larger in amount the urine may have a reddish-brown or a brownish or even a chocolate color, according to the proportion that is present between hæmoglobin and methæmoglobin. Albumin is an almost constant feature, and yet in some fatal cases there may be but traces, and these only on a few days, and alternating with periods of none, even till

death. Serum albumin and serum globulin are present. If many cells are present in the sediment a certain amount of true nucleo-albumin may be expected. Albumose is also present, and in some cases is the only proteid found. The reason for this is not known. It may explain, however, the cases described as albumin-free, the examiner using only a heat test, which did not precipitate the albumose. As a rule, the albumin is not above 1 per cent., and the globulin percentage is relatively high. In the sediment may always be found red blood-cells, mononuclear cells, few polynuclear leucocytes, and epithelial cells from the urinary tubules, which are present singly or in masses, and often very fatty. Among the crystals uric acid and calcium oxalate are found, and hæmoglobin either in amorphous granules or in casts. In the hemorrhagic form of the disease in our series the red blood-cells were evidently remarkably few in number. The leucocytes were very abundant in one case of acute nephritis with multiple abscesses. Casts are present in varying numbers, and may be of any form; epithelial, hyaline, and coarsely granular will predominate, blood and leucocyte casts may also be present. As a rule, the number runs parallel to the amount of albumin, yet it varies greatly from day to day, and on some days may be enormous. In one case of acute hemorrhagic nephritis, with areas of complete necrosis, the amount of albumin was slight but the casts in large numbers, leucocyte and granular. In one case of general septicæmia the albumin was but in traces on certain days, none on others, and yet blood, hyaline, and leucocyte casts were found.

During the course of an acute attack of nephritis the kidney shows every symptom of renal insufficiency. The nitrogen output is diminished, not due to the diet alone. The chlorides and the phosphates are diminished, and hence the molecular concentration is less than normal. The uric acid output is about normal, that of the xanthin bases is said to be increased. The ability of the kidney to form hippuric acid is diminished, and the glycosuria after phlorizin is either slight or absent. In mild cases and in severe ones as they improve the urine approaches normal. It is said that the albumin disappears last, but we believe the casts are often found later.

Nephritis Hæmoglobinurica.—In acute nephritis the amount of hæmoglobin in the urine may be much, the number of red blood-cells few or none. In certain cases this is the cause of the nephritis, in others a symptom. The former is true in cases with blood destruction due to poisons, burns, etc. During infectious diseases the hæmoglobinuria may be secondary, or both that and the nephritis due to the same cause. Such is found in typhoid fever, scarlet fever, malaria, Winckel's disease of the new-born, and other conditions. This form of nephritis differs from pure hæmoglobinuria by the greater amount of albumin

and the richness of the sediment in casts, renal epithelial cells, leucocytes, and uric acid crystals.

Acute Nephritis of Cholera.—This form is said to be a peculiar type of a pure parenchymatous, especially the tubular variety. The urine is diminished even to anuria for from five to seven days even. It is very rich in salts and may have a large urate sediment. Albumin is present in relatively larger amounts than in the other forms of parenchymatous nephritis. The urine is dark and cloudy, rarely bloody Hyaline and granular casts are present; renal epithelium, red blood-cells, leucocytes, uric acid and calcium oxalate crystals. The urine is also characterized by its richness in the ethereal sulphates. Diacetic acid is often present and ammonia is increased. In one case after an anuria of fifteen days the person recovered. The condition of the urine improves much during the stage of reaction.

The Nephritis syphilitica acuta præcox is sometimes marked by the immense amount of albumin present, in one case (Hoffmann and Salkowski) 8.5 per cent. The urine coagulated solid. There was very little sediment, few casts, leucocytes, or blood-cells.

Subacute Nephritis. Chronic Parenchymatous Nephritis. Chronic Diffuse, Non-Indurative Nephritis. Large White Kidney.—This form of subacute nephritis, which may follow an acute attack or develop without this, is characterized by its subacute course, usually fatal within two years, and by the large amount of œdema and effusions in the serous sacs usually present. It occurs especially in young persons, hard workers in exposed, unhygienic surroundings; as a result of constitutional diseases, tuberculosis, lues, malaria; also alcohol. The diagnosis is usually easy from the history.

The amount of urine is always diminished, the diminution varying as the œdema, and especially at death. At the height of the disease it varies from about 250 to 500 cc., as the case improves, however, it increases, and if the patient be encouraged to drink he may void from 5 to 6 litres of a very dilute urine. The amount also is increased as the œdema or the effusions begin to absorb. The specific gravity, varying inversely as the amount, is, as a rule, almost normal or slightly increased, in some cases reaching 1040. The reaction is faintly acid, but in some cases alkaline even on voiding, and in all cases it quickly becomes so. This makes a search for casts difficult. The color is from a pale greenish-yellow to a reddish or a reddish-brown, cloudy as a rule from the large amount of sediment, and foaming easily on shaking because of the amount of albumin that is present.

This is the form in which the albumin is very large in amount, both relatively and absolutely. The amount varies as the specific gravity, roughly, and seems to bear no relation to the œdema. It seldom

reaches 1 per cent., although for months it may vary from 0.4 to 0.8 per cent. In certain cases, however, it is greater. As the case changes to the chronic indurative form the amount of albumin becomes less and less. Cases of 2 per cent. are rare, and Bartels has reported a case which varied from 4 to 6 per cent. The albumin quotient varies much. Nucleo-albumin is present in small amounts, also the albumoses.

The urea is somewhat diminished, even when there is much dropsy. The uric acid varies somewhat less than the urea, and is excreted within normal limits. The ammonia is normal. There is a certain retention of chlorine and phosphoric acid.

The sediments are much the same as in acute nephritis, but it is more common to find coarsely granular, fatty, and waxy casts. Red blood-cells may practically always be found, in especially large numbers in the acute exacerbations. There is little difference between the urine of the white and the mottled kidneys except, perhaps, in the latter there are more red blood-corpuscles, leucocytes, and fatty cells.

Functionally the kidneys are somewhat insufficient, and yet in the severe cases they do their work fairly well. This is thought to be due to the fact that the disease attacks certain successive parts of the kidney, and that while one part is inflamed the other parts can compensate.

One would expect that a glomerular involvement would affect particularly the amount of albumin, the tubular involvement especially the number of casts, and while in general this may be true, clinically it is of little importance, since the two anatomical conditions are always present and variations between them but slight.

Except in young persons with a good past history, the diagnosis cannot be made without an autopsy, since an acute exacerbation of an unsuspected chronic case will show similar clinical features and very similar urine, yet at autopsy small contracted kidneys be found.

Chronic Indurative Nephritis.—A subdivision of this form is exceedingly difficult; in fact, the size and color of the kidney are almost the only criteria, since all its histological elements are affected in nearly every case by degenerative, inflammatory, or regenerative processes. A somewhat related form of nephritis, "senile atrophy," is of almost physiological occurrence in every elderly person, the kidney becoming old and therefore slightly sclerotic with the rest of the body. In fact, in men above middle life Dr. Osler emphasizes the fact that the cortex is never perfectly normal—always a few sclerotic glomeruli and a very slight increase of connective tissue may be found. If the process, however, is simply sclerosis, there should be no urinary symptoms, and hence such cases are not suspected before the autopsy. As a result, however, of hard work, various diseases, gout, lues, and

certain poisons, lead and alcohol, inflammatory processes develop. The result in such a kidney is the degeneration of the epithelial elements, and inflammation resulting in new growth of connective tissue which may be general or focal, subcortical or periglomerular. The kidney becomes hard and firm, diminishes in size, and is finally but a remnant of an organ. In some cases we find contracted kidneys at autopsy which could not possibly have been recognized from the urine. These show that a nephritis limited to foci can heal. In all such cases, however, if it be the sclerotic process which predominates, there may be practically no urinary symptoms, and at autopsy kidneys of surprisingly small size may be found. Other cases are the result of a preceding acute or subacute nephritis.

The only classification which can be made, apart from the weight of the kidney and the thickness of its cortex, is in its color, and hence the division into the red and the white kidney, the red kidneys being the result particularly of arteriosclerosis as a primary factor. The red kidneys are large, firm, beefy, the sclerosis is considerable, and yet the size of the kidneys is seldom as much diminished as in the white form. In all cases it should be borne in mind that arteriosclerosis will be present, in some as the cause of the renal trouble, in others as the result. In a third group, both the renal and the arterial diseases are due to the same cause. The process may be local, one kidney affected more than the other.

Chronic interstitial nephritis in its advanced forms is marked by its very insidious onset. Its only symptom may be a slight albuminuria, and this absent over long periods. Later the albumin becomes permanent, casts appear, and later polyuria. The urine is increased slightly at first, but in a well-developed case from 2 to 3 litres are voided daily, and rarely even as high as 12 litres. On the other hand, it may at times sink to normal or even under. It is pale, clear, definitely acid, and of a specific gravity which is constantly between 1010 and 1005. This low specific gravity in a morning urine is always significant of this condition. The molecular concentration is diminished. The albumin seldom rises above 0.05 per cent., and usually is in mere traces. It is often absent in the morning voiding, and may indeed be present only after a day of unusual exercise or an especially hearty meal or some unusual excitement.

On the other hand, hyaline casts can usually be found on long centrifugalization. Red blood-cells are very common, sometimes hæmaturia. There is often a desquamation of the epithelium cells of the tract, giving a cloudy urine resembling cystitis.

In the **arteriosclerotic kidneys** the urine contains albumin which occurs late and is often intermittent. Cases of the so-called "contracted kidneys with albumin-free urine" belong here, and yet, as a whole,

the albumin is more constant and of larger amount than in the preceding form. In these cases it is the casts which disappear first, in the former the albumin, leaving a cylindruria. No albumin is often seen in the primary contracted kidney; albumin, no casts, in the arteriosclerotic.

The urea is normal, the nitrogen normal, but the percentage of the various nitrogenous bodies may vary somewhat, in uræmia the ammonia rising at the expense of the urea. Uric acid is low and the xanthin bases are increased. The various tests for the functional activities of the kidneys, for instance the methylene blue and the KI test, sometimes indicate a pathological condition and sometimes none. The sediment is scanty and difficult to find. The urine should be centrifugalized and search made over large amounts of urine. After a long search but one or two casts may be found. These are usually hyaline, although sometimes a finely granular. Renal epithelium is sometimes found; a few leucocytes; rarely a red cell, and yet many after overexertion, etc. Uric acid and calcium oxalate crystals are common. It is in this form that the focal character of the nephritis is marked, hence the urine secreted by both the normal and diseased tissue presents the above mixed characteristics.

During acute exacerbations the urine may lose this character entirely, and it be very difficult to distinguish from a more acute form of nephritis. It is well to examine the morning and the evening urines separately, and especially after a day with a particularly hearty meal or severe exercise. And yet, on the whole, the urine in this condition resembles that found in other conditions so nearly that the clinical history and the physical examination of the patient cannot be dispensed with. The urine alone resembles that of acute or the subacute nephritis during convalescence, waxy kidney, and the cyclic, or the so-called physiological albuminurias.

Amyloid degeneration may be superimposed upon any form of nephritis of which it really forms no part. When alone, the urine is said to be normal. In the majority of cases the condition could not be suspected from the urine, although given a case with history and physical signs indicating it, and the urinary changes may be well explained. Without the clinical features the urine would suggest, when concentrated, chronic passive congestion; when dilute, small contracted kidney. The classical description of the urine is an increase in amount, it being pale, clear, faintly acid, of a low specific gravity, 1005 to 1012, abundant albumin as a rule with relatively much globulin, and very few casts. This picture of Traube, however, is rare. The albumin may be little or none, and the casts numerous. These are often fatty. The large numbers of casts which may occur here are not at all distinctive. Renal epithelium is rare, red blood-corpuscles extremely so.

Uræmia.—Uræmia may be considered the highest expression of renal insufficiency, but this is not all. Compensatory changes occur in the kidney, and the body may become tolerant to the toxines, whatever they may be. For this reason it is more common in acute than in chronic nephritis, in which latter case the body has adapted itself to the condition. For these reasons, also, it is easily understood that the urine should show no evidence of an oncoming or present uræmia, for only functional activity can be tested and that imperfectly; resistance never.

But, on the other hand, it is of interest that in cases of anuria due to calculus or removal of the single kidney, uræmia and death may not follow for ten to fourteen days, good evidence that the retention of urinary constituents is not alone enough to explain the condition in uræmia of nephritis without much demonstrable retention.

We have abstracted the histories of 96 cases of nephritis with uræmia. Of the 54 cases in which the first symptom of the nephritis dated back less than six months, 42 died. Of the 35 cases with an old history of nephritis, 29 died. Senator states that the amount of urine is diminished or there is even anuria, rarely does this fail, and more rarely is there polyuria at the onset. The total nitrogen is increased often, but the greatest interest for us at this point is the value in such cases of urea determinations made by the Doremus method. We will not here discuss the method itself. It has been used almost daily as a routine in all severe cases of nephritis. In only eight of these cases of uræmia was urea determination of possible value indicating either its onset by a drop or the improvement of the case by a rise. These may follow, be coincident with, or may precede the change in the symptoms. As the latter was true in but two cases, it is seen how very rarely it is that the oncoming uræmia can be foretold by the urea determination. Of course, in cases of nephritis, as in all conditions, the amount of nitrogen eliminated depends chiefly on the amount of food, and cases with impending uræmia eat poorly, and during uræmia none at all, while the rise as the case improves may be partly due to the increased amount of food. The absolute amount is of no value. The question is, Does its curve rise or fall? It falls no lower in uræmia than in cases of nephritis without it.

There were 13 cases during uræmia with the urea under 1 per cent. We have dealt with percentages since it is almost impossible to get a full twenty-four hours' amount in such cases. Of these 13 cases, the average was 0.74 per cent.; in one case no urea could be determined. Omitting extremes, in 9 cases the urea varied from 0.6 to 0.9 per cent., an average of 0.8 per cent.

In 21 cases in which the urea was no help at all it varied at the onset of the convulsions from 0.9 to 3 per cent., an average of 1.4 and

a mean of 1.4 per cent. The rise in urea in cases with improvement is striking.

In 123 cases of nephritis without uræmia and without much polyuria, in 33 per cent. the urea was at times at or under 1 per cent. In eighteen fatal cases the urea at death varied from 0.3 to 3 per cent., a mean of 1.2 per cent., an average of 1.4 per cent.; that is, exactly the same as in the above uræmic cases.

Uræmia may occur in any variety of nephritis, and with albumin present from a trace to a large amount. It is interesting how little this striking clinical crisis is evidenced in the urine.

One group of ten cases was interesting, since if improvement in condition be indicated by a diminution in albumin and casts with the same output of water, then uræmia may improve a case for a while at least. This of course may not always even suggest improvement, since in two the urea diminished with the albumin and casts, but in four it increased.

In eight cases of terminal uræmia the albumin increased in all. In one case of uræmia with a trace of albumin on the day before and the day following, on the day of the convulsions there was a large amount with a great number of casts.

In *eclampsia* the urinary features are similar to uræmia.

The temporary but extreme albuminuria occurring then is quite striking. In a recent case in Dr. Williams's ward the albumin at 10 A.M., March 6, contained 0.653 per cent. albumin. The woman was in the first stage of labor. Convulsions occurred then. The urine till 5 P.M. of that day contained 1.23 per cent.; that collected till 9 P.M., 0.19 per cent.; at midnight, 0.075 per cent.; 3 A.M., 0.025 per cent.; and March 7, merely a trace. That is, in about twelve hours the output had decreased from 1.20 per cent. to almost the vanishing point.

In another case the total albumin was 0.4678 gms. per 100 cc., and the globulin 0.16 gms. per 100 cc. (34 per cent.). In still another case there were 18 gms. of albumin per litre, a multitude of casts and renal epithelium, yet at autopsy no evidence of severe trouble.

Unilateral Nephritis.—In our series with autopsy no cases of this description occurred. Of 90 cases, in at least 30, or 6 per cent., there was considerable inequality in size of the two organs, yet in all but three cases, or 0.6 per cent., these were large kidneys. In these three cases the combined weights were 155, 190, and 205 gms., and the difference in weight between the two, respectively 45, 50, and 65 gms. In a very interesting case at operation was found unilateral suppurative nephritis.

Renal Atrophy.—This may be due to insufficient blood-supply, to cachexia, the anæmias, and especially to advancing age, the "senile atrophy." It is never great in amount. There are seen microscopically

sclerosed glomeruli, but no great increase in connective tissue. The urine is practically normal, and without albumin.

Congenital Cystic Kidney.—The urine in this very rare condition may be normal, or show the picture of the chronic interstitial nephritis with small contracted kidney. The amount of urine is increased, the specific gravity low, with or without a trace of albumin, often much blood. The contents of these cysts are rather interesting, being not at all uniform, and in the same kidney different cysts may have different contents; sometimes clear, watery, almost colorless, or milky or colloidal; sometimes containing urea, even in large amounts, or uric acid, sometimes none. Often cholesterin crystals: colloid or proteid-like masses, rosette masses which resemble leucin have been described.

Suppurative Nephritis.—In such cases we have the ordinary symptoms of acute nephritis with albumin of varying amounts, but only a few casts. In the sediment, however, in one case there were a great many red blood-cells and leucocytes. In the other cases there seem to have been very few leucocytes. When many, the urine will be alkaline. Very rarely fragments of renal tissue have been found.

Dr. Kelly had recently on his service a remarkable case of unilateral suppurative nephritis.

In cases of purulent nephritis the amount of pus which is found may be disappointingly small since the kidney with the abscess, either as a whole or in the affected part, may excrete no urine. In the metastatic renal abscesses there are no urinary symptoms as a rule.

Cancer of the Kidney.—Hæmaturia is often an early, even the first, symptom. It occurs in over one-half of the cases, and is the first symptom in one-fourth. It may be from a very slight trace to a fatal hemorrhage, intermittent or of long duration, the blood fresh or decomposed, and clots even of large size may be voided. Otherwise the urine is practically normal.

Tuberculosis of the Kidney.—In a general miliary tuberculosis there are no urinary symptoms as a rule, and when present are not due to the tuberculosis alone. In tuberculosis of the pyramids, in which case it is common to have large caseous masses which break down and leave a cavity, the so-called "renal phthisis," the urine is similar to that in pyelonephritis. If it be the pelvis which is involved, caseous matter may be found in the urine. If the pelvis be normal there may be no urinary changes. The very early polyuria with or without albuminuria is an interesting feature. Hæmaturia may be the first symptom, and was present in eight of the seventeen cases from this clinic which were reported by Dr. Walker.[163] This is early, even the first symptom, not great in amount, and may continue for months. It is present both day and night, and bears no relation to the position

[163] Johns Hopkins Hosp. Rep., vol. xii.

of the patient, hence differs from that due to calculus. On the other hand it may be so severe as to be a serious feature. Pus was present in fifteen of the seventeen cases, in little or large amount according to the position of the cavity. Blood-clots are common; tissue detritus and masses about the size of a grain of sand occur, and in them are found tubercle bacilli and elastic tissue. These were present in nine of the seventeen cases. Albumin was present in sixteen, and casts in six of this series. On the other hand, the urine may for days be perfectly normal, and then present all the above mentioned features, the reason being that during this period no urine was excreted by the diseased side.

In general, it may be said that in all cases of hæmaturia and pyuria, especially with acid urine, tuberculosis of the kidney should be excluded. For diagnosis the tubercle bacilli must themselves be found. But even this is not enough, since there is much evidence for the Cohnheim idea of the "excretion" of these bacilli through a practically normal kidney, hence tuberculosis of other organs must also be excluded. If the disease remain limited to the parenchyma of the organ the entire kidney may be destroyed and yet be unsuspected.

In **infarction of the kidney** there is usually a preceding nephritis. In the sediment red blood-cells are usually present, but marked hæmaturia is rare.

In cases of bilateral infarcts, there may be oliguria, even anuria. An intense albuminuria with sudden onset and rapid disappearance and no abnormal sediment is a very suggestive feature.

Pyelitis and Pyelonephritis.—Inflammation of the pelvis of the kidney may be due (1) to an infection ascending along the ureter, or a descending renal infection, or an infection extending by contiguity from neighboring organs; (2) to local causes, stone, cancer, tuberculosis, parasites (echinococcus, amœbæ, etc.), trauma, floating kidney; or (3) to systemic causes, specific toxines of acute fevers, medicines, etc. It is usually unilateral.

The symptoms are usually masked by those of the general or causative disease, and even when attention is directed to it there may be no localizing symptoms.

The urinary features will depend on the cause. Sometimes there is anuria (due to the reflex influence over the sound kidney). In chronic cases the amount of urine is sometimes even trebled. It is cloudy from the pus, blood, and mucus, and faintly acid unless there is ammoniacal decomposition. It contains little albumin unless nephritis coexist.

Microscopically, the urine contains red blood-cells, mucus, pus, various epithelial cells, uric acid crystals, calcium oxalate crystals, fibrin coagula, tissue constituents, and other elements suggesting the

cause of the trouble, as tumor fragments or parasites. It has been a much disputed question whether from the nature of the epithelial cells the situation of the trouble could be determined. It seems to be generally conceded that the epithelium from the pelvis of the kidney to the urethra is quite uniform. Sahli suggests from observation of one case that in pyelitis it is the cylindrical cells with tails (Fig. 46, a, b) which are especially increased. We have found many of these cells in several cases, but in one very acute case with autopsy the urine contained none. The epithelial cells are often in clusters with strata, presenting the well-known tile arrangement of the tailed club-shaped cells. There will be no casts or renal epithelial cells in case there is no nephritis. In the diagnosis the urine examination is particularly important. The reaction of the urine is usually acid.

Of importance is the variability of the urine, the obstruction of the diseased side causing periods with normal urine, then the appearance of all the elements of the pyelitis.

Various crystals are present, and in the diphtheritic form threads of fibrin and casts of the pelvis of the kidney, or tissue fragments.

In the diagnosis of pyelitis, of greatest importance is the absence of disturbance of micturition, the homogeneous mixture of pus and urine, and the club-shaped tailed cells in groups with a tile-like arrangement.

In **hydronephrosis, pyonephrosis,** and **uronephrosis,** the urinary changes (apart from pus) are in amount of urine, the periods of oliguria alternating with polyuria, and its constituents, depending on the health of the cortex.

Renal Calculus.—During the renal colic the urine may be normal or anuria total, but when the obstruction is relieved, blood, mucus, and pus appear.

Independent of colic, hæmaturia is a common symptom (especially of oxalate stones), sometimes with the passage of a clot of blood; sometimes the hemorrhage is profuse, especially early. Later the symptoms are those of pyelitis.

With *ureteral calculi* occur hæmaturia and oliguria, followed by polyuria. The oliguria is a feature in about 25 per cent. of all cases, and even anuria in 16 per cent.[164]

Parasitic Diseases of the Kidney.—In *echinococcus disease* the only urinary symptoms are in some cases a mucous catarrh of the pelvis of the kidney, which later may be purulent pyelitis or gangrene. If the large cyst ruptures through the urinary tract, there is the sudden appearance of a watery fluid (or soapy or milky or bloody), and while the cyst is discharging the hooklets, scolices, fragments of membrane, etc., may be found in the sediment.

[164] See Schenck, Johns Hopkins Hosp. Rep., vol. x. p. 477.

(For other parasites see page 275.) In the *Bilharzia infections* (see page 275) pyelitis and even renal atrophy may result.

FUNCTIONAL RENAL DIAGNOSIS

During the past few years the amount of work in this very promising field has been enormous (over fifty theses and articles have appeared in about seven years), but, sad to say, is rather unfruitful.

The discrepancy between the anatomical condition as found at autopsy and that which would be supposed from the urinary examinations is proverbial. Small contracted kidneys of less than one-half or one-third normal size may excrete a urine normal in amount and specific gravity, with but a trace of albumin and a few casts; when the urine was full of casts and a great amount of albumin, no clear evidence of nephritis may be found; again, persons may die with the most marked signs of renal insufficiency, uræmia, with a urine almost normal, albumin but a trace, and at autopsy very slight renal changes. Evidently the time-honored chemical and microscopical methods are too gross.

The next problem was to find a more delicate test than these to determine a renal condition which would be unsuspected if ordinary tests were used, and which would also allow of diagnosis before the anatomical changes were evident; also a test which would prophesy an oncoming uræmia. Two of the most delicate tests of physical chemistry were chosen,—cryoscopy and electrical conductivity of the urine and blood. It was hoped that the results of this work would be more in accord with pathological findings on the one side, and when these would be deceptive could we see the kidney, would show a definite renal insufficiency did it exist clinically. In connection with these tests is often used the sodium chloride test.

The third line of work disregarded the anatomical condition of the kidney altogether, and asked as to its functional ability. For, a well-compensated severe lesion is manifestly of less immediate danger than a poorly or non-compensated slight lesion. In these tests an extra demand is suddenly made on the kidneys and its response determined. Such tests are the sodium chloride, the methylene blue, the rosanilin, the phlorizin, salicylic acid, and potassium iodide, *et al.*, tests.

Before describing these tests it is well to emphasize the fact that the toxines so evident from their results in nephritis are as yet unknown; and that nephritis is more a general disease, the kidney features playing only one part. All that can be done is to test the way in which the kidneys behave toward known substances, or perform their ordinary duties, or the unusual which we impose upon them, and from this by analogy surmise how well they perform their other

functions. Another point to be remembered is that the function of the kidney is not as well understood by the physiologist as the clinician seems to assume when he uses the methylene blue test to test the "epithelial filter," the salicylic acid to test the "glomerular filter," and phlorizin to test the "glandular activity" of the renal epithelium.

Most admit that one test is never enough; that all must be used to get a good picture, and even with all one is dissatisfied (see the many French theses of 1902 to 1903, among them Miorgec, Lyon, 1902, and Jouffray, Lyon, 1903).

We wish to warn workers that nearly all these functional tests make an unusual demand on the kidneys, to which they may not be able to respond, and disastrous results follow.

Cryoscopy, Freezing Point of the Urine.—By means of this determination one hopes to find out how many molecules the kidneys are excreting on the one hand, how well they keep the serum free from an accumulation of these molecules on the other. Of these two values the latter is the more important, since the former will depend on the diet, etc. The latter is an index of the success the kidneys have in keeping the plasma free from the products of catabolism. It is not what the kidneys eliminate, but what they should but do not, which it is of interest to determine, and the examination of the blood gives some clue as to this.

The determination of the freezing point of solutions is a well recognized method of physical chemistry to determine the molecular weight of the substance in solution; also the degree of disassociation of the molecules. The method, therefore, is, from the chemist's point of view, one of great importance. But even when the problems are of the simplest nature, when dilute solutions of a single and pure salt are used, and with but the simplest point to determine, the method requires experience, skill, and the due regard to a good many factors which can modify the results. It is hard to see, therefore, how this method can be applied with much success to complex fluids like the urine or blood, in which is dissolved a great variety of bodies of widely different nature, some unknown. The belief was, however, that from very slight differences in the freezing point of these fluids important deductions could be made concerning the functional ability of the kidney. It must, of course, be remembered that the changes in the freezing point are exceedingly slight, varying in many cases but a few hundredths of one degree; a variation which the physical chemist considers slight is that from which the clinicians draw conclusions. The clinician, therefore, should use even greater care than the physicist in using this method, and at least as delicate instruments. The reverse has, however, been true. Cheap "clinical" instruments which

cannot be accurate are placed upon the market, and a multitude of freezing point determinations made with a disregard of the chances of error which must exclude the possibility of correct results even in a much simpler problem.

The principle of cryoscopy is this: When a substance is dissolved in a liquid, the freezing point of the latter is lowered to a degree which in a general way is proportional to the concentration of the solution. The lowering of the freezing point of a given liquid, for instance of water, by a 1 per cent. solution of a substance, is called the "specific depression" of the freezing point of that liquid by that particular substance, and a 2 per cent. aqueous solution of that same substance should cause double that depression. It has been found that the depression of the freezing point bears no relation to the molecular weights of the substance, but to the number of molecules in solution. In other words, equal numbers of molecules when dissolved in equal quantities of a given liquid will produce the same lowering of the freezing point whether the molecules are of the same or different nature. By "molecular depression" is meant that caused by a solution in 100 gms. of a given liquid of a number of grammes of the substance in question equal to its molecular weight.

The above statements have one very important exception. They hold for those bodies which are not disassociated in solution. In the case of electrolytes—inorganic acids, bases, and salts—in dilute solution the depression of the freezing point is at least twice that of equivalent amounts of organic substances, since electrolytes are disassociated, and each ion has the same effect on the freezing point as a complete molecule. The difficulties are at once evident. In the blood and in the urine we will have a large number of bodies in solution in proportions varying considerably, probably in conditions of association which we can neither control nor determine. The result obtained by the clinical chemist is therefore a depression of the freezing point produced by a resultant in this mixed solution of really unknown nature.

Of course, this result could be of greatest value if it were empirically shown to be so, even though the phenomenon could not be well explained.

The question is, then, Does experience prove cryoscopy a valuable clinical means of determining renal sufficiency, or is the clinician only playing with a scientific toy which has for him only the appearance of truth?

Method.—The apparatus (see Fig. 62) consists of an exceedingly delicate thermometer, *a*, and the best is none too good, which reaches almost to the bottom of a test-tube, *b*, scrupulously clean, containing the solution whose freezing point is to be determined. This

test-tube is enclosed in an air-jacket, *c*, which is surrounded by a freezing mixture of ice and salt.

The Beckmann thermometer is generally used. The makers of this instrument now supply one for freezing points alone, which is a distinct advantage over the Beckmann for both freezing and boiling points. In the freezing mixture is inserted a smaller thermometer, *d*, that its temperature may be controlled, and a stirrer, *e*, that it may be kept well mixed. Before and after every determination the zero of the thermometer must be determined for distilled water. Of the same water the second zero will often be slightly lower than the first since the freezing has purified the water of carbon dioxide. The lower of these values is the zero of the experiment.

The fluid whose freezing point is to be determined must cover the whole or at least over two-thirds of the bulb of the thermometer. In any accurate apparatus this will require at least 10 cc. of fluid, and those instruments advertised for 5 cc. are to be regarded with great suspicion. The temperature of the freezing mixture should not be more than 1° to 3° lower than the freezing point of the fluid to be determined, since undercooling may give too low a point.

The method used by the physical chemist is as follows: Distilled water is put into the tube, *b*. The water is allowed to cool somewhat below the freezing point and then by means of the stirrer, *f*, stirred vigorously until ice begins to form. The thermometer will then rise a little and remain con-

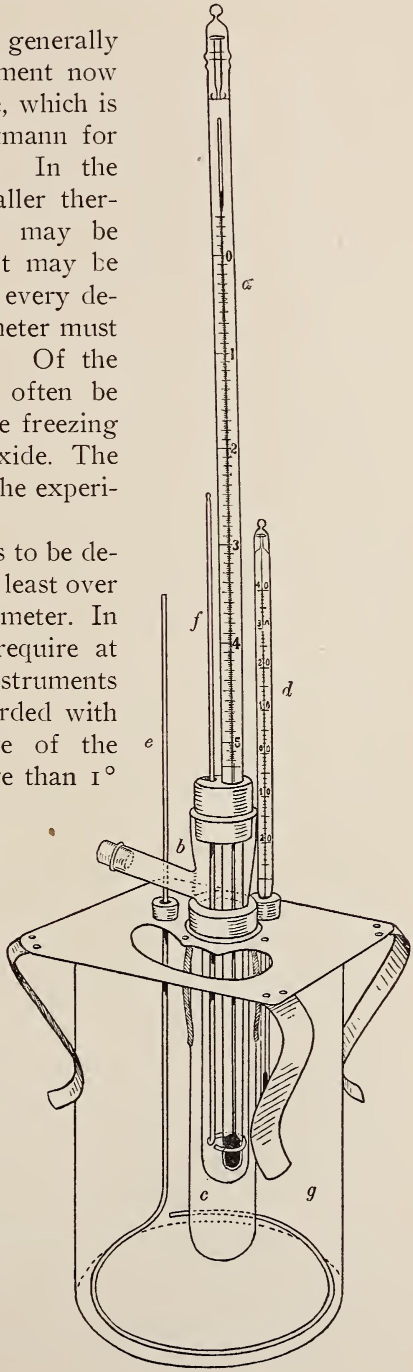


FIG. 62.—Apparatus for freezing-point determinations. *a*, thermometer graduated to 0.01° C.; *b*, tube for fluid to be investigated; *c*, air-chamber; *d*, thermometer for freezing mixture; *e*, stirrer for freezing mixture; *f*, stirrer for fluid; *g*, jar for freezing mixture.

stant. The highest temperature is the true freezing point of the water. This is to be repeated several times and the lowest used. The test-tube containing the water is then removed and that containing the fluid in question substituted. This also is stirred until ice begins to form, at which time the thermometer will rise, remain stationary for a few moments, and then fall. The highest temperature is the one to be recorded. The rise is caused by the liberation of the latent heat of crystallization, and the subsequent fall due to the concentration of the solution, since the ice formation removes just so much solvent. That this highest point may be the correct one it is necessary that the freezing mixture should not be too cool, or we may get "under cooling." If ice does not form, a very small crystal of ice should be inserted into the tube, which will start the freezing at once.

Certain points must be observed with care: The repeated correction of the zero point for each determination already mentioned. The bulb of the thermometer would better not rest on the bottom of the test-tube, or there may be too much ice formed here; this error is, however, small. The stirrer should theoretically be moved by machinery, since its motion should be perfectly rhythmical and timed by a metronome; irregular stirring results in error. Since the cooling should be slow and the stirring take fifteen to thirty minutes, it is impossible to do this by hand.

But this careful performance of the test is seldom carried out. As a rule, the clinician considers the determination easy and remarkably rapid, and for him it is, but we are glad to say that long series of observations have been made by those who have observed all the above precautions, and their work (mentioned later) is alone of value in judging the method.

Some go so far to the other extreme as to say that the molecular concentration may be easily determined by multiplying the last two figures of specific gravity by 0.075.

To determine the freezing point of *urine* it is better to use a twenty-four hours' mixed specimen than to test separate voidings. The vessels in which this is kept should be perfectly clean. If rich in urates the freezing point of the cloudy fluid may be determined, and to the result 0.04° added or the fluid may be cleared by centrifugalization and the freezing point of the clear fluid and sediment determined separately.

Linossier and Lemoine [165] have shown that the position of the patient is important, since excretion is very different when erect from when in bed, being even five times as much in the latter position.

Blood.—This is best obtained through a canula in the median vein at the bend of the elbow. One must be sure, however, that the cir-

[165] Compt.-rend. Soc. Biol., vol. lv. pp. 469, 605.

culation is perfectly free before the blood begins to flow, since the venous stasis alters the freezing point considerably. Serum, defibrinated blood, or the pure blood may be used with theoretically the same results. Most of the better workers, however, prefer the serum. The defibrinated blood has the error of the high CO_2-content which can make a difference of 0.02°. It is better to let the blood clot in an air-tight vessel, or to condense the clot by centrifugalization. At least 40 cc. of blood in the first case are necessary in order to get enough serum; in the second case, from 25 to 30 cc. Two determinations should be made, the observer being sure that the serum is perfectly thawed between them.

In the following paragraphs δ = the depression of the freezing point of blood; Δ that of urine.

The results obtained were at first considered promising, but more recently the method has not received the same approval. Koranyi, who brought the method into prominence in 1896, was exceedingly sanguine. He considered that δ was very little lowered, that is, below 0.56° C., in anæmias and fevers which do not affect respiration, but was lowered considerably by any disease causing insufficiency of respiration, or renal insufficiency, or both. He thought that cryoscopy would help the diagnosis between typhoid fever and pneumonia, since in the latter δ is lowered. In renal disease, for Δ to be high (*i.e.*, abnormally near 0°), means renal insufficiency. From cryoscopy of the urine he believed in anæmias Δ to be less than 1.4°, a point which is considered normal. In nephritis it is very little less, but in anæmias, in renal insufficiency, and in malnutrition is molecular oliguria present (about —0.80° C.). It is seen that he expected much from this method.

The freezing point of normal serum in man is much less variable than that of any other animal, it being from —0.55° to —0.57° C. In case both kidneys of an animal be removed, the effect on the blood is enormous, even—0.75° C., but a partial injury has a less effect, Koranyi stating that one-half the renal parenchyma could be destroyed before any effect was shown.

The remarkable ability of the body to keep δ constant is seen in the case mentioned by Kümmel, in which δ was —0.57° C. before an intravenous injection of 2000 cc., and four hours later exactly the same. This writer in a long series of observations found almost always when there was unilateral disease a lowering of δ.

The amount of work done with cryoscopy is enormous, at first quite promising, but later less so. Our own experience is not sufcient to report. We think the present status well given by Schoenborn (Wiesbaden, 1904), who has done some very careful work with this method. Forty-two cases of nephritis were studied and

the various formulæ applied. His conclusions are that the ordinary methods of urine examination, the microscopical and chemical, are of more value than cryoscopy of the urine and blood; that clinically the latter can be omitted but the former never. He found that from cryoscopy no information was gained concerning the nature of the processes, the functional ability of the kidney, or the severity of the disease. It is true that the majority of cases will conform to certain rules, but one is so often deceived, the number of exceptions to these general rules so great, and in all probability their number will increase so greatly on further work, that the value of this alone is to be much doubted. As a delicate method of predicting any future change of the patients' condition or of detecting a latent nephritis which cannot be recognized by the ordinary clinical methods, he doubts that it is of any value.

In most of his cases Δ fell within those limits usually considered normal, and some of these were the severe ones. In the severest cases δ did drop, yet gave no indication of the nature of the lesion.

In uræmia it is granted there is the most serious renal insufficiency, and hence this is the condition by which the method can be best tested. He concluded from the study of 88 cases, including nine of his own, that cryoscopy in some cases shows a normal condition, in the majority of cases the same condition as of nephritis without uræmia, and in only a few cases a striking abnormality, as, for instance, $\delta = -0.975°$ C. Engelmann on the contrary reported a series of 36 cases, δ averaging $-0.664°$ C. Again, in cases with no suspicion of renal insufficiency δ could be very high, even $-0.67°$ C.

In surgical cases with a gross bilateral renal lesion destroying all the functions a good parallelism between the lowering of δ and the development of the uræmic symptoms may be followed (Kümmel), but in medical cases this is not so true, since the lesion, whatever it may be, in some way brings about uræmia while those renal functions which we can measure are still well performed.

According to the medical men, therefore, the cryoscopy of the blood and urine gives some idea of the osmotic activity of the kidneys which is not given by any other method; this idea enlarges in some cases the clinical picture and is of some value, but it rarely gives any information which could not be learned from the ordinary methods. It never foretells an oncoming uræmia, or decides the nature, the prognosis, or the results of therapy, or reveals a condition not already suspected. It is a method which requires a great deal of practice, and the accurate control of a great many conditions, both in its performance and in the previous care of the patients, such as diet, etc. The results obtained by the skilful are interesting, in some cases valuable, in the majority disappointing, and in some deceptive. The cryoscopy of the

blood alone demands considerable experience, and gives little more than would be expected from the clinical observation of the case, and often not that much. The cryoscopy of the urine is of very little value even when greatest care is taken concerning diets, fluid, etc. Schoenborn examined 52 cases of non-nephritics with many diseases, and found it of practically no value whatever in differential diagnosis or to follow the results of therapy. He admits that the conditions of the kidney and cortical vessel are the most important factors governing δ and Δ, but there must be other unknown factors in some cases still more potent. From the study of all these cases no general deduction could be made.

Strauss,[166] who carefully governed the food and water intake, also had found cryoscopy of the urine unsatisfactory, and evidently more cases atypical than typical. For the value of cryoscopy in hepatic diseases, see Ferrannini.[167] Cryoscopy has also been used for the quantitative determination of albumin and sugar, but without much success.

Among the various FORMULÆ used in the hope of getting constants for use are the following:

Δ = depression of freezing-point of urine; δ, that of blood.

$\frac{\Delta \times \text{amt. of urine (V)}}{\text{body weight (P)}}$ (Claude and Balthazard) "the total molecular diuresis;" the index of "glomerular filtration;" this was found inconstant.

$\Delta \times$ amt. of urine, Strauss' "valence value," is useful; the "molecular diuresis" of Koranyi.

$\frac{\Delta \times \text{amt. of urine}}{61.3}$ "NaCl equivalent" of Koranyi (Δ of 1 per cent. NaCl = 0.613).

$\frac{\Delta}{\text{N per cent.}}$ (Waldvogel).

$\frac{\Delta}{\text{Sp. gr. of urine.}}$

$\frac{\Delta}{\text{NaCl}}$ (Koranyi).

$\frac{\Delta}{\delta}$ is of value indicating the permeability of the epithelium of the tubules.

$\frac{\Delta \times \text{amt. of urine}}{\delta}$ (Bernard).

$\frac{\delta\ \text{V}}{\text{P}}$ an approximate index of urinary toxicity.

Electrical Conductivity.—This method of physical chemistry also has been appropriated by the clinician in his desire to learn something

[166] Zeitschr. f. klin. Med., 1902, vol. xlvii. p. 39.
[167] Centralbl. f. inn. Med., vol. xxiv. p. 273.

about the functional ability of the kidneys. By electrical conductivity is meant the reciprocal of the resistance which a certain amount of a solution between two platinum electrodes of given size and given distance apart offers to the passage of a current of known strength. This is really a measure of the number of electrolytes in solution, that is, of the disassociated ions. It is not affected by such bodies as albumin, sugar, urea, which are not disassociated, and hence is practically a measure of a few salts in the blood and urine, especially the chlorides. This also is a method which is very valuable to the physical chemist of experience to determine simple points concerning simple solutions, and even for him to draw deductions requires a full understanding of all the conditions present in the determination. It would seem, therefore, like one working in darkness to apply this very delicate method to solutions, such as the blood and urine, which contain an unknown mixture of various bodies which, because of this mixture, may not disassociate as they would were they in pure dilute solution. Yet if found by experience to be of value, no theoretical objection should be urged against it.

Method.—The method of Kohlrausch is that usually used. By this method an alternating current is passed between platinum electrodes through the solution whose conductivity it is desired to study. The resistance is balanced on a Wheatstone bridge against a rheostat, and the point of equilibrium determined by means of a telephone. The urine, for instance, is placed in a U-shaped tube of known length and holding 4 to 8 cc. of fluid, in which are immersed the platinum electrodes, which must be very carefully prepared and covered with platinum black in order to secure a sharper minimum in the telephone. This vessel containing the urine is placed in a thermostat, the temperature of which does not vary over 0.1° C.

For detailed description of the method standard works on physical chemistry should be consulted. But this method has been popularized, and on the market are instruments for "clinical use" which allow of a rapid determination and, which is their chief advantage, require a very small amount of urine (1 to 2 cc.).

The conclusions thus far are that the electrical conductivity in the case of the blood and the urine is somewhat parallel to the freezing point and gives nothing definite. Many abnormalities are found, but one does not know how to interpret them. For this reason we refrain from a more elaborate description of the method.

The delayed excretion of urea is an old criterion of functional renal ability. In acute disease it may take three to six days to excrete the urea formed from one day's meals; in chronic nephritis and renal tuberculosis, two days. During this delay the urea accumulates in the blood, and may be determined quantitatively (see page

513). When it is increased tenfold (*i. e.*, to 0.3 per cent.), there is danger of uræmia (Herter).

Chloride Excretion.—The rapidity with which the kidneys can excrete a considerable amount of sodium chloride is taken as a test of its functional ability, or, as some specify more particularly, of the "glomerular sufficiency."

This test of "alimentary chloruria," recommended by Claude and Manté,[168] is used usually in conjunction with cryoscopy. It consists in placing the patient on a milk diet (3 l. per day), and after the third day adding sodium chloride, 10 gms. a day, dissolved in 125 cc. of water, which are given in three portions on each of four consecutive days. The daily output of the chlorides is determined.

Normally the excretion begins at once and ends abruptly when ingestion ceases. The water output is increased, but to a less degree. The other constituents of the urine are also slightly increased and continue so for a longer time than the increase in Cl.

These writers divide renal cases into four groups: the first, of those who react exactly as normal persons do; these bear their lesion well. In the second group the increased chloride excretion causes a considerable increase of other urinary constituents, especially the nitrogenous bodies, which continues several days, as if the sodium chloride had acted favorably on the kidneys. In the third group the increase in chlorides is delayed, reaches a slow maximum, and continues two to four days after ingestion ceases. There is also an increased output of other bodies, but less than in the second group. In the last group the ingestion of salt causes no increased Cl output, none or a slight diuresis results, and the excretion of other bodies is increased. The prognosis of the third group is bad; of the fourth, fatal.

Yet further work has shown that there is nothing constant in this excretion in renal disease; there are too many exceptions, and not always can these be interpreted.

The Dilution Test.—Some consider the change of Δ after ingestion of increased fluid of importance. In parenchymatous nephritis the ability to excrete a dilute urine is decreased more than it is in contracted kidney; that is, the case is judged from its water economy. In surgical cases also this test is considered a valuable adjunct. After determining Δ, etc., the patient drinks in a short time 1 to 2 litres of water. The amount excreted and the Δ of the dilute urine are then determined. The urine is collected in half-hourly amounts. The increase often begins during the second half-hour, reaches a maximum in two to three hours, and is over in five to six hours.

Among the other terms used are "glomerular insufficiency," which means the retention of water and NaCl, the excretion of a scanty concentrated urine, the inability to excrete a dilute urine. "Tubular insufficiency," the retention of nitrogen and phosphates, the excretion of a normal amount of dilute urine, the inability to excrete a concentrated urine.

[168] Arch. gén. de méd., 1902, vol. viii., n. s., p. 129.

Renal Permeability.—THE METHYLENE BLUE TEST (of Achard and Castaigne) is supposed to test the "epithelial filtration." This dye may be given by mouth, 0.1 gm. in a capsule, or 0.05 gm. subcutaneously, that is, intramuscularly (1 cc. of a 1 : 20 solution of methylene blue). This latter is the better method, since the disturbing factors on the part of the digestive canal are eliminated.

The dye is eliminated, first as a colorless chromogen in fifteen to thirty minutes after a subcutaneous injection, and as a greenish-blue pigment three to five minutes later. To appear first only after an hour is pathological. Normally the excretion reaches a maximum in from three to four hours and lasts from thirty-five to fifty hours (forty-eight to sixty); the chromogen is last to disappear. About one-half is eliminated in the first twenty-four hours.

As soon as given the bladder is emptied and the urine examined at stated periods, first in half an hour, then hourly. It is boiled with acetic acid to oxidize the chromogen. The time is measured from the appearance of the colorless pigment thus made evident until this chromogen disappears.

It has been noted that toward evening the elimination ceases, to be resumed the following morning. Others show a "polycyclic" elimination, *i.e.*, a very irregular curve with periods in which none is eliminated. This is seen in interstitial nephritis and various neurotic conditions, but is said to be especially true of "hepatic insufficiency" (Pugnat and Revilliod).

But the delay or non-delay of elimination is not of great importance, since even a small amount of normal renal tissue in an extensively diseased kidney will excrete some at the normal time, hence the amount excreted is considered of greater value. The results of these tests of the renal permeability in the hands of its friends, especially the French, may be stated as follows: The delayed and protracted excretion depends directly on the acuteness of the process; in chronic parenchymatous nephritis, as a rule, the permeability is good; also in some cases of chronic interstitial nephritis. Others say that in nephritis the excretion is always delayed; that when elimination begins in normal time either the kidneys are sound or the lesion local.

In some cases the excretion is delayed and lasts longer than normal, even seven days, and the total output is less. This is seen in renal atrophy, and is considered a sign of "diminution of the excretory surface" (Achard). In some cases "with epithelial lesion predominating" the kidneys seem abnormally pervious, the excretion begins in a very few minutes, and continues but about twenty-four hours (Bard). In a third group it begins late, but lasts only a short time; *e.g.*, Widal's case began at the end of five hours and lasted but two hours. The greatest abnormality is therefore in interstitial nephritis, while

in parenchymatous or amyloid the test may show a normal or abnormally rapid output.

Herter, while admitting that a delay means disease, agrees that some patients show periods of normal output. He denies that in any case is there a shortened period of excretion.

From the first the test has been severely criticised (especially by Germans). Too many cases with kidneys found at autopsy to be the seat of extensive disease reacted normally or with abnormal rapidity. In uræmia even the test may show normal permeability (but see Bard's case). The variations are not marked enough; it is not reasonable to judge of the permeability of the kidneys to normal or abnormal constituents from the excretion of this very abnormal body, and since the permeability for methylene blue is known to be different from that for known bodies, it may be very different from that for the toxine causing uræmic symptoms; while, on the whole, in nephritis there is delayed and abnormally protracted excretion, yet the various forms of renal disease show little or no difference; the permeability is altered even in neuroses. In some cases the dye is entirely destroyed in the body, not even the chromogen appearing in the urine. And lastly, even the warmest friends of the test have modified it, and now emphasize the total output of the dye as of most importance, which causes one to mistrust the test, for this quantitative determination is uncertain, hard, and it is not at all certain that this amount would be any good index of renal activity.

Salicylic Acid Test.—This test was adapted to clinical use by Widal and Ravant as a measure of renal permeability. One cc. of a 30 per cent. solution of sodium salicylate is injected (with a little cocaine to reduce the pain) intramuscularly. The urine is examined at the end of half an hour, then hourly, with 10 per cent. Fe_2Cl_6 solution. Colorimetrically can the amount excreted be determined.

Normally the violet color is given by the urine voided at the end of half an hour, even of fifteen minutes; it reaches a maximum in from one to three hours, and disappears in from eight to twelve hours. The amount excreted (*i.e.*, the per cent. of the total) in five hours is taken as a sort of standard. The excretion is supposed to be through the glomeruli and governed by physical laws alone. In the various forms of nephritis the excretion may begin within the first half-hour (yet perhaps in the first fifteen minutes), and reach a maximum at the same time for all. In some of the cases of parenchymatous nephritis there is the same duration of excretion and the same relative output in five hours as normal, but in some interstitial cases the output is continued over a long time and is less in amount; yet in even the very few cases reported there are striking exceptions.

It has certain advantages over the similar POTASSIUM IODIDE test, since it is simpler and more rapid.

If a QUANTITATIVE ESTIMATION be desired, Ziegan[169] recommends the following: To from 30 to 50 cc. of urine in a graduated mixing cylinder are added 1 cc. of dilute H_2SO_4 and 50 to 80 cc. of ether, and shaken three to five minutes. This is allowed to settle. One-half the ether extract (with the salicyluric acid) is removed by a pipette and poured into a separating funnel. To it is added 2 per cent. Fe_2Cl_6, till the color does not change. It is then poured into a glass suitable for color determinations. Into another glass of exactly the same character is poured an equal volume of ether, the same amount of 2 per cent. Fe_2Cl_6 which was added to the first glass, and then 0.1 per cent. of salicylic acid from a burette until the same shade is obtained.

For the quantitative estimation of KI see Singer.[170]

Phlorizin Test.—This test of the "secretory ability" of the renal epithelium, rather than the "permeability," for which latter function osmosis is supposed to play the important part, in the former none, was proposed by Achard to replace the HIPPURIC ACID TEST (the ability of the kidney to transform benzoic to hippuric acid; a theoretically good test of renal functional ability, but clinically useless since the determination of hippuric acid is so inexact). The test is, of course, based on the generally accepted view that phlorizin diabetes is due to the activity of the renal cells and that a diminished or absent glycosuria means renal disease. One cc. of a 1 : 200 solution of phlorizin (hence 0.005 gm.) is injected subcutaneously (a small dose is chosen which will produce glycosuria in only normal kidneys). The bladder is emptied, then sugar tested at intervals. In normal persons sugar is evident in one-half to one hour, and it lasts two to four hours. The quantity eliminated is 0.5 to 2.5 gms. of glucose. In nephritis, as a rule the sugar is below 0.5 gm. or there is none. Abnormalities in the test may not always indicate renal lesion but more or less functional disturbance of that organ. The test does not permit to separate the various forms of nephritis, the "hypoglosuria," and "analglycosuria" occurring about equally in all forms. Yet it is a good test of renal activity and tests a quite different function than the others.[171]

The Value of these Tests to the Surgeon.—In the medical wards these tests may be said to add to the clinical pictures of cases, but for diagnosis not much weight is given them. For surgical conditions the case is very different, and these tests are of great value, when, *e.g.*, the

[169] Centralbl. f. inn. Med., 1903.
[170] Zeits. f. klin. Med., 1903, vol. xlviii. p. 157.
[171] Pugnat and Revilliod, Arch. gén. de méd., 1902, vol. viii. p. 19.

question is the justification of removing a diseased kidney. In such a case the first question is, the presence of another kidney; the second is, can this second kidney do the work of both? To decide these questions, if by means of ureteral catheterization we can separate the urines excreted at the same time, a comparison of these is of value in determining, first, the presence of the second kidney; second, the relative values of their activity; while the freezing point of the blood will determine their united insufficiency; *i.e.*, as in medical cases, if lowered, a contraindication to operation. It is of interest that the confidence in these tests of the medical men has decreased, of the surgeons increased.

The chief difference between these two points of view may be that surgical cases are chiefly of renal disease which destroys all renal function, while among the medical cases there are so many in which all the functions which can be tested are normal, but that unknown but all-important one, failure of which means uræmia, escapes detection. Kümmel[172] stated that he had in a long experience (in over 500 cases) never been deceived by cryoscopy of the blood, and Casper and Richer[173] consider the cryoscopy of the separated urines, and the phlorizin test to determine the amount of sugar eliminated by each kidney, neither test alone but the agreement of both, of actually greater value than the microscopic or gross examination of the renal tissue. The surgeons take it for granted that the secretion of two normal kidneys at the same time is quite equal. (Although it may vary much at different times, even in eight minutes reaching in normal persons figures which would be considered pathological, hence the collection from the two must be simultaneous. Casper and Richer.) Others think that for short intervals the renal activity is scarcely equal, and that at least two-hour specimens should be collected to be sure of an equality. The kidney can be said to be insufficient, when Δ is less than $-1°$ C., unless the urine be diluted by recent intake of fluid.

The methylene blue test is of less value, since one cannot well separate the urines for so long a time as would be necessary to determine beginning, end, and intensity of output; yet the surgeons do use it to test whether the fluid from a sinus is urine, *e.g.;* the phlorizin test is more valuable, since one can determine these three points by letting the catheters remain in the ureters but three hours, but even that is not necessary, since the difference in amount of sugar excreted by the two kidneys simultaneously is an index of the relative amount of renal activity on the two sides; since normally their excretion is equal. The urine should be examined by the polariscope in from one-half to one

[172] Centralbl. f. Chir., 1903, vol. xxx. II. p. 110; also Arch. f. klin. Chir. Bd. 67.

[173] Functional Diagnosis of Kidney Disease, 1903.

hour after the injection. Barth,[174] who used the method of Casper and Richer, says that examination of the separated urines gives not an absolute, but a relative picture of the functional ability of the two kidneys.

Göbell [175] also determines only Δ, and warns one that he cannot trust these functional tests implicitly. The patient should be for several days on a constant diet, the ureters catheterized at the same time after a meal, and the catheters left in place two to three hours to collect the urine. From Δ one cannot tell whether or not the remaining kidney will be sufficient.

The dilution test has proved of very great, some say the most, value to the surgeon, the diseased kidney not responding as well as the other. The catheters are left in place for from three to five hours. The urine is first examined, the patient drinks 1.5 to 2 litres of water, and the voiding for each kidney is examined especially with regard to amount and Δ.

The increased output on the normal side may begin during the second half-hour and reach a maximum in from two to three hours; the diseased side may show no increase.[176]

Kümmel, on the other hand, considers the freezing point of the blood more important, and mentions 72 cases of nephrectomy without a mistake in judgment concerning the sufficiency of the second kidney, whether sound or slightly diseased. As a proof of the value of cryoscopy he states that before its use mortality of renal surgery was 28 per cent.; since its use, 8 per cent.; and of nephrectomy, 4.8 per cent.

Kümmel found in those cases in which there was an unconfirmed suspicion of renal trouble a constant δ of 0.56°, with 0.54° to 0.58° as limits. (These figures all refer to depressions of the freezing point. When one says $\delta = 0.56°$ C. he means that the freezing point was depressed that much, that is, that the temperature of the freezing mixture was —0.56° C., or $t° = -0.56°$ C.) In the second group was disturbance of total renal function, bilateral nephritis, pyelonephritis, etc., with $\delta = 0.60°$ to 0.65°, limits 0.59° to 0.81°; in these surgical cases of uræmia (prostatic cases *e.g.*) was a definite parallelism between the molecular concentration and the uræmic symptoms. In a long interesting series of cases of other than renal disease he finds δ practically normal. He says that for him 0.6° is the limit of safety. If below this limit, although the one kidney may not be strictly normal, yet it is sufficiently so to do the work of both. The third group is a long series of cases of unilateral disease; when strictly unilateral, in all δ was normal. In such cases ureteral catheterization showed on the affected side Δ low and urea diminished, while the other side was

[174] Centralbl. f. Chir., 1903, vol. xxx. II. p. 134.
[175] Münch. med. Wochenschr., 1903.
[176] Illyés and Kovesi, Berl. klin. Wochenschr., 1902, p. 321.

normal. In cases of apparently unilateral disease with δ normal, in no case did subsequent history show that there had been a bilateral disease. Cryoscopy may also be used in differential diagnosis; *e.g.,* if the question lies between renal calculus and hemorrhagic nephritis with unilateral pain, a high δ would speak for the latter; in prostatic hypertrophy the presence or absence of an ascending disease can be settled by determining δ; that a tumor is renal can be suspected by the low Δ of that side.

CHAPTER III

THE STOMACH CONTENTS

THE VOMITUS AND GASTRIC CONTENTS

THE various forms of vomiting have been grouped as follows:

Cerebral: in brain and cord disease, as tabes, insular sclerosis, meningitis of brain or cord, cerebral anæmia or hyperæmia, concussion of the brain, brain tumors, etc.

Toxic: opium, tobacco, ether, chloroform, alcohol, uræmia, cholæmia, pregnancy, *et al.*

Psychical: disgust, fright, anger, and other strong emotions.

Periodic: "cyclic," "recurrent," a form with sudden attacks of vomiting, often without apparent cause, and sometimes accompanied by intermittent hyperchlorhydria. There is evidence that some of these cases, especially of children, and which resemble a secretory neurosis, are due to an acidosis, *i.e.*, an autointoxication.[1]

Neurasthenia and hysteria; especially the latter: In an interesting case of very obstinate vomiting of neurasthenia lately in the ward, the woman repeatedly, three to four hours after the stomach was washed out, would vomit three to four ounces of bile-stained fluid.

Reflex: as in peritonitis, strangulation of the bowel, sexual disturbances, cholelithiasis, renal colic, intestinal worms.

Local: due to gastric conditions, whether acute or chronic, and especially those with stasis of the gastric contents.

The Vomitus and General Considerations concerning the Gastric Contents.—Considerable may be learned from vomitus, yet less than from a test meal. The gross inspection is valuable, its microscopical examination less so, and its chemical often misleading, for we seldom know the condition of the stomach previous to the meal which is vomited, nor always the character of this meal, at least we are sure it was of no standard quality and amount; the time element cannot be controlled, and it contains mucus and saliva from the mouth. Our test meal analyses, conducted with the greatest of care observing all of these points, are none too satisfactory, hence the examination of vomitus apart from its gross appearance is even less so.

The REACTION of vomitus, with the exception of a few cases of achylia, those with intestinal contents mixed, and a few cases of cancer of the stomach with alkaline gastric contents, is acid to litmus. If free hydrochloric acid is present it is an important point to exclude fluid from diverticula of the œsophagus.

[1] Edsall: Snow, Am. Jour. Med. Sci., 1904, vol. cxxviii.

The **character** of the vomitus is important; abundant, thin, acid fluid, with food eaten the previous day, means dilated stomach; very fluid, strongly acid juice free from food, means continuous secretion; thin acid fluid, with finely divided fragments of food, occurs in ulcer; thick masses, with much mucus and poorly digested, often decomposing meat, occur in catarrh and cancer of the stomach; almost undigested food in nervous vomiting. If the vomiting occurs at the height of digestion and during a paroxysm of pain which at once diminishes, ulcer; if during or shortly after eating, cancer, catarrh, or a neurosis; independent of eating, also mornings before breakfast, and of not only mucus and bile but also food remnants, ectasis. Cerebral vomiting is often marked by a noticeable absence of straining or effort; vomiting on rising in the morning is suggestive of pregnancy, or, in the case of men, of alcoholism; with cancer at the cardiac orifice, vomiting follows a meal; at the pylorus or pyloric stenosis due to any other cause, later, and at longer intervals, and large in amount.

A very slight BLOOD streaking of vomitus and gastric contents is of no moment, since from the effort of vomiting or by the stomach-tube slight lesions of the œsophagus or pharynx may result.

BILE AND PANCREATIC SECRETION.—Traces of bile are often present in vomitus from a fasting stomach, at the end of lavage, and in vomiting attended by severe retching. This has no significance unless it be constantly present and there has been no straining sufficient to force bile from the duodenum into the stomach. In case it is constantly present, it might indicate stricture of the duodenum below the ampulla. On the other hand, a green color does not always indicate bile, since a few cases are recorded [2] of "grass green," "sea green," "dark green" vomitus, the color of which is due to algæ or at least to chlorophyll-colored protophytes. Bile-stained vomitus was particularly common from an almost empty stomach, less so from a full in which case there would be more counterpressure against the pylorus, preventing the regurgitation of bile. For this reason it was thought the vomitus of peritonitis was more often bile-stained than of cerebral troubles, since in the former cases the stomach is more often empty.

MUCUS in the vomitus is almost constant. Seldom, however, does it indicate gastric catarrh, a condition which is rare compared with the number of times that the diagnosis is made. It may be due to catarrh, but more often to lack of hydrochloric acid and hence lack of digestion of the mucus that is normally secreted. A vomitus of only mucus, or one containing large amounts, is seen in the morning vomitus of alcoholics.

Large amounts of acid gastric juice, sometimes pure, sometimes mixed with food, are common in cases of hypersecretion, the former

[2] See Kuhm, Zeitschr. f. inn. Med., 1902, No. 28; 1903, No. 1.

especially in cases of gastroxynsis, a neurosis with periodic attacks of acid vomiting. If food be present in this case the proteid will be well digested, the starch less so.

The vomiting of large amounts in which is food eaten two or three days previously occurs in cases of stricture of the pylorus with dilatation of the stomach. Of this vomitus the proteid will be poorly digested and badly fermented in case of cancer, etc., well digested with fermentation of carbohydrates in benign cases due to ulcer, etc., this depending on the presence or absence of hydrochloric acid.

Fecal vomiting results from complete obstruction of the ileum or the colon, and in peritonitis or other condition with paralysis of the intestinal wall. The successive vomitus are more and more fecal, and hence it is easy to say approximately from what part of the intestine each comes, until finally we have black, foul-smelling contents of the colon, which microscopically contains vast numbers of bacteria. Yet the absence of fecal vomiting does not always exclude a total obstruction, as when it is high in the jejunum, for to have even a suggestive fecal odor the obstruction must be at least six feet from the pylorus.

Rice-water vomitus is seen in Asiatic cholera. It is very fluid and filled with white flakes of mucous shreds and epithelial cells (see page 343).

From the *color* and the *odor* of the vomitus cases of poisoning or alcoholism may be suspected. In uræmic cases it has an ammoniacal odor.

Some idea of the *motility* of the stomach may also be obtained, since if any food is vomited seven hours or more after the last meal, motility is certainly diminished, although this may be a very temporary condition. At the end of two or three hours particles of meat should be swollen and show considerable evidence of digestion. At the end of one hour bread should have been broken up to a fine, crumbly sediment, which settles to the bottom of the glass. If there are large particles of bread, and especially if these are coated with mucus, hydrochloric acid is quite surely diminished.

The chemical analysis of vomitus, as stated above, is exceedingly unsatisfactory. If free hydrochloric acid be present we are sure that it is secreted, but, if absent, we can draw no conclusions. In general, it may be said that normally both free hydrochloric acid and pepsin are present two hours after a mixed meal.

Lactic acid may be expected after a mixed meal.

The microscopical examination is also unsatisfactory, since normally both intact muscle-fibres and starch granules pass to the intestine.

The *antiseptic condition* of the stomach may also be judged. The presence of organisms may be due either to the absence of hydrochloric

acid or to stasis. The vomitus may be foamy and have the odor of butyric or other organic acids. In cases with free hydrochloric acid and severe stasis the majority of organisms are yeasts and sarcinæ; in lighter cases of stasis the fluid is sterile; in those cases, as of cancer, without free hydrochloric acid, bacteria predominate.

TUMOR FRAGMENTS occur, but are rarely found. In the vomitus may be found round worms, segments of tape-worm, oxyuris, maggots, etc.

Examination of the Fasting Stomach.—While from the normal fasting stomach theoretically no fluid should be obtained through the stomach tube, yet it is very common to get from 10 to 50 cc. of an acid gastric juice. There is considerable discussion as to what amount is certainly abnormal, and the limit given by Boas of 100 cc. is a safe one. This much means hypersecretion or motor insufficiency. The question may be settled by washing the stomach out at night; if due to motor insufficiency, the stomach will be empty in the morning. Riegel insists that the fasting stomach is always empty, even a little true gastric juice is pathological, and that the few cubic centimetres found is not normal digestive fluid.

THE FLUID FROM THE FASTING STOMACH is thin, specific gravity 1004 to 1005, contains some free hydrochloric acid, no lactic acid, and no bacteria. It is very commonly bile-stained, but this is not important unless repeatedly so, in which case duodenal stricture may be suspected. It may be alkaline from the presence of pancreatic juice, in which case trypsin should be tested (see page 354). Should the fluid be neutral or even acid from hydrochloric acid, soda must be added at once to prevent the destruction of the trypsin. The presence of abnormal amounts of mucus may be determined, such as occurs in anacidity, atrophy of the mucous membrane, etc., but considerable washing is necessary to dislodge the mucus from the mucosa.

Test Meals.—THE EWALD-BOAS TEST BREAKFAST consists of white bread, about 40 gms., water or tea without sugar or cream, about 400 cc. The bread should be chewed very fine. This breakfast is to be removed in just one hour. Usually 30 to 70 cc. are obtained, of specific gravity 1012 to 1020. If 200 to 300 cc. are gained, there is hypersecretion, motor insufficiency, or perhaps disturbed absorption.

RIEGEL'S meal consists of one plate of beef soup, from 150 to 200 gms. of beefsteak, and 150 gms. of mashed potatoes. It is to be removed in from three to four hours.

Riegel and others have emphasized that a test meal should be one to which the patient is accustomed. For this reason, in Germany the breakfast consists of bread and tea or water, and the test meal of beef etc., since this is a fair sample of the diet to which the German laborer is used. Both Ewald and Riegel also insist that the meal should be

given at that time of the day at which the patient is accustomed to ingest a meal of that character. But in this country such meals, particularly the Ewald breakfast, are not customary, are not in the least like ours, and yet American observers quite uniformly use this breakfast, overlooking the fact that one cannot test normal physiological phenomena with abnormal meals.

Again, meals should be chosen with reference to the case. Not only does the average fare differ in different countries and in different grades of society, but the important individual peculiarities of taste and habit cannot be totally disregarded. In adopting these two German meals we therefore entirely neglect the two important points,—that the meal should be one to which the patient is accustomed, and given at the hour at which he is accustomed to take it. It is small wonder that many are sceptical as to the value of their use. But the work of physiologists (Pawlow) has shown that in the secretion of gastric juice the psychical element—that is, the influence of taste, sight and smell, etc.—is almost equal to the chemical element,—that is, the stimulus from the absorption of soluble products of gastric digestion,—and since the Ewald breakfast is certainly unpalatable, it is perhaps valuable for that reason, since it rules out in some measure the former factor, and while to find diminished acidity may not mean much, yet hyperacidity does mean that there is certainly some trouble present. Many Americans appreciating these facts have attempted to introduce meals for American patients. Of course, no two persons' tastes are alike, yet we can select as standard a meal more like our patients' diet than the above. Fischer's meal is perhaps as good as any, which consists of the bread and water of the Ewald breakfast plus a quarter of a pound of finely chopped lean beef broiled and slightly seasoned. It is to be removed in three hours.

Fischer has shown by comparing results with his meal with those of the Ewald breakfast that those with his are much more constant than with the latter. For instance, repeated examinations were made with the Ewald breakfast; in 40 per cent. of the cases the findings with the later meals were quite different from those with the earlier. With his meal there was need of changing the diagnosis in but about 8 per cent. of the cases. Using both meals in the same cases, in 67 per cent. they gave similar results, 18 per cent. of those showing hyperacidity with the Ewald meal showed less with his meal, and in 15 per cent. more. Of the subacidity cases with the Ewald, 30 per cent. were normal by his. In various clinics, we have noted that several meals were used, the Ewald meal giving some idea of what the stomach will do when an indifferent meal is given which excites but little secretion, the Riegel indicating the possibilities when there is greater tax upon the secretory cells. Some can handle the test breakfast well, but cannot the larger meal, while others respond well only to the greater stimulus. Fischer gives several points which might aid in differential diagnosis based on the use of two meals. Concerning the diagnosis of the anatomical lesion he says that if the stomach be subacid to the breakfast but normal after a proteid meal, we can state that the secretory structures are normal, and may suspect that atony with the constant

presence of food has rendered the mucosa less sensitive; if subacid to the proteid meal as well as to the breakfast, it indicates organic changes; if subacid to the breakfast but hyperacid with a proteid meal, it could mean defective innervation; the same is true if hyperacid to the breakfast and normal to the larger meal; if the hyperacidity of the breakfast continues and increases with the proteid meal one may suspect a probable increase of oxyntic cells, since the secretion is not in proportion to the stimulus, especially if the secretion continues several hours after the meal. If the symptoms and the increased secretion both diminish after the meal, disturbed innervation may be suspected. He also emphasizes the fact that certain cases of dyspepsia which have been on an almost starvation diet for some time need to be fed up pretty well before a test meal is given. This may cause a gastric upset, but the flare-up of the condition will be an advantage in the diagnosis.

In all cases we should be sure that the stomach is empty before the meal is given. This may be done in the case of the breakfast by lavage the preceding night, unless experience teaches us that there is in this case no motor insufficiency. The patient should get used to the meal and to the tube, and hence the first meal is seldom of any value, and the second should be confirmed by at least one other. Another point on which sufficient emphasis has never been laid is that the meal should be removed at the time of optimum secretion. The Ewald test breakfast should not always be removed at the end of sixty minutes, nor the Riegel at the end of five hours. With the Ewald breakfast, as is easily seen by the study of cases in which meals are removed at different intervals, in some cases with rapid motility the maximum acidity is attained in forty minutes, in others in an hour and a quarter. In either case if the meal be removed at the end of just an hour, an erroneous idea will be gained by the low acidities found. Another point particularly important in neurotic cases is that the time at which the meal is given should be chosen with reference to the symptoms, since at other times than during the nervous disturbances the gastric condition may be normal.

Many other meals have been proposed, *e.g.*, the whites of two hard-boiled eggs and 100 cc. of water (Jaworski and Gluzinski), and much more complicated ones (Pfaundler-Sahli, *et al.*). We have tried nutrose to some extent, in the hope that the pure proteid meal would teach more concerning the products of proteid digestion. This meal is not at all appetizing, and was given up.

While it has long been recognized that we were foolishly fitting to the American stomach European meals, and need new standards following the use of American meals, yet in a general way much has been learned from these two meals.

Acidity of the Gastric Juice.—The fluid removed after the test meal or breakfast should be first tested with litmus. This will indicate acidity in general, which may be due to hydrochloric acid free or bound, to organic acids should they be present, and to acid salts. In the great majority of cases the litmus will show acid; in a very

few cases the fluid is alkaline. In a recent case of cancer in these wards the fresh fluid was alkaline to litmus. It contained many pus-cells. The next point is the presence of free acid, and Congo red is the indicator usually used, indicating free organic or inorganic acid.

The tests for FREE HYDROCHLORIC ACID are all of them color-tests. The first and perhaps the commonest used, since it is the easiest, is the above-mentioned Congo-red paper. Free hydrochloric acid will turn this to a sharp blue, while free organic acids, even in strong concentration, will give a much less definite blue. A trained eye usually has no difficulty in recognizing from this test alone whether it be free hydrochloric acid or free lactic acid that is present. Acid salts, if strong, give a positive test with Congo-red, but they do not occur in this concentration in the stomach.

Methyl violet is the indicator first suggested by V. d. Velden, who first showed the presence of free hydrochloric acid in the gastric juice.[3] This is a very satisfactory test. One drop of the saturated aqueous or alcoholic solution of methyl violet is mixed with water in a test-tube until the color is a pale violet. This is divided in two test-tubes; to the one is added the filtered gastric juice, to the other the same amount of water. Free hydrochloric acid will turn the violet to a fine blue color. It requires a much larger amount of organic acid. This indicates 0.025 per cent. free hydrochloric acid.

Tropæolin OO has been used, but is less sensitive than the above, indicating 0.03 per cent. In this case the saturated solution is used in the same way as the methyl violet. If free hydrochloric acid is present, the yellow is turned to a reddish-yellow color. Boas suggests that this be used as a contact test, a few drops of the concentrated solution of the stain being warmed in a porcelain dish and brought into contact with a small amount of the gastric juice. The dish is then warmed over a small flame, and if free hydrochloric acid be present a fine violet or true blue color is formed.

Dimethylamidoazobenzol is most commonly used, and is a very sensitive indicator. It gives a pink color with free hydrochloric acid, but it reacts also to organic acids and acid phosphates in concentrations which might occur in the stomach.

Günzberg's solution (Phloroglucin, 2; vanillin, 1; alcohol, 30) is the standard test. One or two drops of this solution (which should be kept in a tightly corked blue bottle) are warmed on a porcelain dish until just dry. One drop of the gastric juice is then allowed to come into contact with this and the warming continued. If free acid is present, at the edge of contact will appear a beautiful crimson line. This was considered very sensitive, showing 0.005 per cent. of the free hydrochloric acid. Now, some (*e.g.*, Unterberg) says it is less sensitive than others. It is of value, since it indicates nothing but free inorganic acids. The test, although so easy, is often spoiled, since the solution is burned by too much heating and a brown non-characteristic color apppears. The fluid should not be allowed to get too old.

[3] Deutsch. Arch. f. klin. Med., Bd. 23.

It is to be emphasized that the above color-tests are for free hydrochloric acid; that is, for the acid in excess of all acid binding bodies, such as proteids, hexone bases, etc.

Free acid is present in the stomach after a carbohydrate meal in from one-half to three-quarters of an hour; after meat, from one to one and one-half hours; and after milk and potatoes in three-quarters of an hour.

Total Acidity.—This is the starting-point in all gastric analyses. This figure represents the highest amount of hydrochloric acid which could possibly be present, and, compared with the amount of free acid, gives a good picture of the secretory function and the motility of the stomach.

Acid bodies which could be present are the free and the bound hydrochloric acid, other acids, as lactic, butyric, etc., and acid salts.

To 10 cc. of filtered gastric juice is added an indicator. Tenth-normal NaOH is then added from a burette, stirring the fluid all the time until the first change of color is seen throughout the whole volume. This titration may be done in a porcelain dish, a beaker, or an Erlenmeyer flask, the latter two against a white background.

The indicator usually used is phenolphthalein, two or three drops of a 0.5 per cent. alcohol solution. Others have been proposed, among them litmus, cochineal, methyl orange. Phenolphthalein is preferred because of the sharpness of its reaction, yet it is perhaps the worst indicator possible, since this sharp end reaction does not always indicate the point of neutralization of all the acid elements, especially since ammonia is sometimes present in no small amount. The reason for its continued use is the desire for comparable results to which an empirical value may be given. The same gastric juice will give very different results with different indicators, and those with this are too high.

Some use the filtered gastric juice, some the unfiltered, shaking it up well to a homogeneous suspension, since the solid particles contain relatively more of the acid than the fluid portions.

Two methods of expression of results are in vogue; the one to estimate from the amount of sodium hydroxide used the equivalent amount of hydrochloric acid. The number of cubic centimetres of the sodium hydroxide used multiplied by 0.00365 gm. equals the amount of this acid by weight in 10 cc. In the case of total acidity this would be the highest possible amount of hydrochloric acid which could be present, hence has the advantage of stating the outer limit of possibility, although a certain amount of total acidity is surely not due to hydrochloric acid. As an exact statement of truth the suggestion of Jaworski is usually followed; that is, the number of cubic centimetres of the alkali which would be required to neutralize 100 cc. of the

gastric juice, and to this the term "acidity per cent." is applied. Since 10 cc. are usually used, the titration figure multiplied by 10 will give the acidity per cent. without reference to the acids which may be present.

As illustration: if, using phenolphthalein as indicator, 10 cc. of the juice required 8 cc. of tenth-normal NaOH to neutralize the acids present, the acidity per cent. would be 80. Supposing that HCl were the only acid present, then the gastric juice would contain 0.29 per cent. HCl. To avoid confusion, the symbol of percentage is never used for "acidity per cent."

TABLE OF EQUIVALENTS.

Acidity per Cent.	Gravimetric per Cent.
10	0.0365
14	0.05
20	0.073
27	0.1
34	0.125
40	0.146
48	0.175
50	0.182
55	0.2
61	0.225
70	0.25
73	0.275
80	0.292
87	0.317
90	0.329
95	0.347
100	0.365
105	0.383
109	0.4

Free Hydrochloric Acid, Mintz Method.—Ten cc. of the filtered gastric juice are titrated with tenth-normal NaOH until the test for free acid is no longer positive. This is based on the supposition that the NaOH will neutralize the free before the bound HCl.

Of indicators there are several. Undoubtedly the most accurate is the Günzberg. As the sodium hydroxide is added, small drops of the stirred fluid are removed by a glass rod, or, better still, a platinum oesa, and tested on a porcelain dish (see page 314). Fleiner adds 25 to 30 drops of the Günzberg reagent directly to the gastric juice, and then, as the sodium hydroxide is added, removes small drops which he warms in a porcelain spoon. Sahli recommends that the glass rods themselves with which the soda is stirred into the gastric juice be warmed, since the crimson color can be seen on the rod. He also adds from 25 to 30 drops directly to the fluid. In this method a certain amount of gastric juice is lost in each of the tests, and hence the results should be confirmed by a new portion from which less is removed.

A much easier method, and one that is chiefly used in some clinics

in which the best gastric work is done, is to add the sodium hydroxide until small drops touched by a rod to Congo-red paper no longer turn this blue. Some find approximately the end reaction with Congo-red, and then more definitely with the Günzberg. The Congo-red should be used as a paper moistened by drops removed from the fluid, rather than added as a solution to the fluid, since with free acid results a suspension of a bluish-black precipitate which makes the end reaction difficult. As little should be removed as possible for each test, and the color produced by the drop controlled by one with distilled water.

Töpfer Method.—The method in quite common use in this country, since it is the quickest, employs dimethylamidoazobenzol as the indicator. The smallest drop possible, in fact, a small fraction of a drop from the end of a glass rod, is added to the gastric juice, which will take a bright red color. The sodium hydroxide is now added until the red element of the color is lost. The end reaction requires a trained eye, since the transition from bright red to clear yellow is a broad one, with the important point the disappearance of the red shade.

The amounts of free acid determined by these three indicators are by no means the same, as the difference sometimes amounts to 100 per cent. Günzberg will always be the lowest and dimethylamidoazobenzol usually the highest. Congo-red paper varies very much, some qualities giving results almost as low as Günzberg, some the highest of all.[4]

Hydrochloric Acid Deficit.—Tenth-normal HCl is added to 10 cc. of the gastric juice until the test for free acid is positive. The amount necessary will depend on the amount of bound HCl already present, the amount of proteid and bases to bind the acid, and the amount of alkali secreted, hence a better term suggested by Sahli is the "saturation deficit." Congo-red paper or Günzberg can be used, but the former is sufficiently delicate. The determination of the HCl deficit is quite as important as of the free acid, since the progress of the case either downward or toward improvement can thus be followed.

For the **bound hydrochloric acid** Töpfer recommends alizarin as indicator. This now is little used.

Fischer,[5] after neutralizing with tenth-normal NaOH for total acidity, then adds an amount of tenth-normal HCl equal to that of the alkali added, and then a 4 per cent. calcium phosphotungstate solution to 30 cc. It is allowed to stand three to four minutes, animal charcoal added, and filtered. To a measured part of the filtrate 6 drops of 1 per cent. rosolic acid are added, stopping at a deep orange tint, and its acidity determined. All the proteid has thus been precipitated, and the bound HCl left as $CaCl_2$. The total acidity minus that of the filtrate equals the combined acid. In case no free acid is present, the deficit is

[4] See Johns Hopkins Hosp. Bull., January, 1903.

[5] Am. Jour. Med. Sci., 1903, vol. cxxvi.

first determined, then the total acidity. Enough tenth-normal HCl is then added to raise the total acidity at least 40 per cent., then one precipitates the proteid with calcium phosphotungstate and proceeds as above. A simple calculation gives the bound acid.

Total Hydrochloric Acid.—Hydrochloric acid is present free, bound, and as neutral chlorides. Of the latter we have those from the food, those formed in the stomach, and those secreted as such.

By the total hydrochloric acid is understood the bound and the free ("the physiologically active hydrochloric acid"). The Lütke-Martius method is one of the simplest for determining this. The principle on which this is based is that the difference between the total chlorine ($= a$) and the chlorine after incineration ($= b$) represents that volatilized by heat; *i.e.*, the hydrochloric acid. This method has been corrected by Reissner, who showed that with the HCl, NH_4Cl is also volatilized. He, therefore, first neutralizes the gastric juice with tenth-normal NaOH, using litmus as indicator. This neutralized fluid is then ashed and the chlorine determined ($= a'$).

$$a - b = HCl + NH_4Cl,\ a - a' = NH_4Cl,\ a' - b = HCl.$$

The Arnold and Lütke methods are used to determine chlorides. (For the solutions necessary, see page 123.)

Determination of "a."—Ten cc. of the gastric fluid are measured with a pipette into a flask with a 100 cc. mark on its neck. Twenty cc. of Solution 1 are then added, stirred, and allowed to rest for ten minutes. A few drops of 8 per cent. $KMnO_4$ are then added if necessary to decolorize. The flask is then filled with water to the 100 cc. point and the contents well mixed. The fluid is then filtered through a dry filter until one-half has passed through. Fifty cc. of the filtrate are measured into a beaker and Solution 2 added from a burette till the first permanent brown.

The number of cubic centimetres of Solution 2 necessary to precipitate the excess of silver are then multiplied by 2, since but half the fluid was used in this titration. This product, subtracted from the amount of $AgNO_3$ originally added, will give the amount of $AgNO_3$ used in precipitating the chlorine.

Determination of "b."—Ten cc. of the gastric juice are evaporated to dryness on a water-bath in a platinum dish. This is then burned over the free flame until the ash no longer burns with a luminous flame. It is not brought to a red heat, since this would volatilize some of the chlorides. The ash is then rubbed up well with water by a glass rod, extracted with about 100 cc. of warm water, brought onto the filter and washed until a few drops of the filtrate no longer give a precipitate with $AgNO_3$. To the whole filtrate are then added ten cc. of Solution 1, and the determination proceeds as for "a."

Determination of "a'."—Other ten cc. are first neutralized with tenth-normal NaOH, using litmus as indicator, then ashed and the remaining chlorides determined as for "b."

$(a'—b)$ 0.0365 gm. = the per cent. of HCl.

Topfer's Method.—This method promised to be a very simple and useful one, since it was purely volumetric and could be finished in a very few minutes. The stomach contents were titrated with dimethylamidoazobenzol for the free hydrochloric acid and with a 1 per cent. aqueous solution of alizarin for the bound hydrochloric acid, the sum

of these being the total hydrochloric acid. This method received severe criticism at once, since both indicators were not above criticism and the end reactions rather doubtful, yet Hari in Boas's laboratory found it quite as accurate as some of the more elaborate methods if free hydrochloric acid is present; if absent, inaccurate.

Absolute Amount of Hydrochloric Acid Secreted.—It is of course evident that the preceding methods give merely percentage values, the percentage of the acid in the stomach contents at that time, and without reference to the total amount of acid at other times present. The absolute amount of hydrochloric acid at a stated time has been a matter of considerable investigation, especially by Bourget and Geigel.

The last method proposed is the following, the preceding methods being all rather tedious. The stomach-tube is introduced and as much of the gastric juice expressed as possible. Then 300 cc. of water are allowed to flow in and out of the stomach several times. From the difference in the specific gravity of these two fractions the amount of undiluted gastric juice in the second fraction can be computed. The acid of the first fraction is then determined and that of the second calculated and added to it. This method has some scientific, but no practical value, since considerable of the acid secreted has already passed into the intestine.

Leo's method for **total acidity** is based on the principle that all acids whether free or bound are neutralized by $CaCO_3$ while acid salts and acid binding bodies remain unchanged. To 10 cc. of the gastric juice are added 5 cc. of concentric $CaCl_2$ solution (since acid phosphates may be present), and titrated with tenth-normal NaOH, using phenolphthalein as the indicator. Let the number of cubic centimetres used be "*a*."

Fifteen cc. of gastric juice are rubbed up with 1 gm. of pulverized and dry $CaCO_3$, and filtered through an ash-free filter. Of the filtrate, 10 cc. are freed from CO_2 by a stream of air, and then 5 cc. of $CaCl_2$ solution added, and this fluid titrated as above (result $=b$).

$a - b$ equals the total acid. If no organic acid be present, this is all HCl.

The Value of the Tests for Acidity of the Stomach.—Among the above tests the simple presence or absence of free hydrochloric acid is of the greatest importance, much more so than is its percentage amount. Practically every stomach can secrete some acid, but the normal stomach will always secrete a physiological excess. It may be that the bound acid is alone of value in digestion of proteid, but it is the amount free which is the index of the functional ability. The percentage of the free acid is of importance in determining the question and grade of hyperacidity, and the deficit to determine the grade of the case and to follow it in its improvement. It is to be remembered that accurate quantitative work requiring considerable time is not justified, for only percentages can be determined, since the total amount can never be obtained. The point of departure is the determination of total acidity. If the Ewald test breakfast is used the phosphates are unimportant, and hence the acidity is the sum of the hydrochloric and organic acids. These are never present together free and if free hydrochloric is present the organic acids can be disregarded. Knowing these few points, valuable deductions can be drawn. For instance (quoting Sahli), if the total acidity be high and no free hydrochloric

present, the acidity is due for the most part to free organic acids. If the lactic acid test be good or the odor of the other organic acids perceived and many bacteria are present, this diagnosis is confirmed. If free hydrochloric is present and the total acidity low, the most will be due to hydrochloric acid, and the motility of the stomach may be assumed to be good, since the acid binding bodies have passed on into the duodenum. If, on the other hand, the total acidity be moderate, and free acid small in amount, a poor motility may be assumed, with the retention of the products of digestion.

Physiology of the Gastric Juice.—After the ingestion of a test meal secretion of gastric juice begins almost at once. All the hydrochloric acid is at first bound. By the end of a half-hour after the test breakfast or two hours after the Riegel meal, as a rule, enough has been secreted so that some remains free. The amount of acid rises to a maximum, and then as the products of digestion pass on into the intestine the acidity begins to fall. One hour after the Ewald breakfast the total acidity averages normally 40 to 60 acidity per cent., or 0.15 to 0.22 per cent. HCl; over 0.25 per cent. means hyperacidity (some say 0.2 per cent.). Lactic acid is not present. Acid phosphates are no factor. The free HCl is from 20 to 60 acidity per cent. or 0.05 to 0.2 per cent.; the bound from 0.012 to 0.11 per cent. Although this, as a rule, is at sixty minutes from the meal, yet the optimum will depend on the motility of the stomach, in some cases having reached this at forty minutes and in other cases at one and a quarter hours. It is important, therefore, to seek the optimum of each case before drawing any conclusions, or a mistaken idea of the amount of secretion may be gained.

With the Riegel meal the normal total acidity is about 75, but from 90 to 100 acidity, and free about 44 may be present.

Diagnostic Value.—Sahli gives the following very valuable summary:

A. There is normal acid secretion: (1) Often in ulcer of the stomach and stenosis due to the contraction of its scar; (2) gastric neuroses; and (3) simple atony.

B. Hydrochloric acid is increased, that is, the free more than 0.2 per cent., and total than 70 acidity per cent. (it may reach 0.35 per cent. and very rarely 0.8 per cent.): (1) In the majority of cases of ulcer of the stomach; (2) in true continuous hypersecretion (but not the hypersecretion due to motor stasis); (3) simple hyperacidity and hypersecretion occur only during digestion at which time the per cent. of acid is abnormally high; (4) paroxysmal hypersecretion (gastroxynsis) occurring in neurotic individuals, who, following some excitement or other disturbance, vomit large amounts of acid juice; (5) in some cases of chlorosis (in 22 of 30 of Riegel's cases); (6) early

stages of chronic gastric catarrh; (7) many forms of mental disorders.

C. The hydrochloric acid secretion is diminished in (1) fevers; (2) severe anæmias; (3) the majority of cases of chronic gastric catarrh; (4) many gastric disorders due to general neuroses; (5) many forms of mental disease; (6) after long standing jaundice; (7); many chronic cachexias, as tuberculosis of the lung, but not always; (8) chronic passive congestion due to heart disease or to emphysema, etc.; (9) sometimes in chronic nephritis; (10) after the long use of alkaline and saline purges.

D. Free hydrochloric acid is absent on several examinations (and yet the stomach always contains a certain amount of this acid bound) in all conditions under C of a severe grade; such as amyloid disease of the stomach, toxic gastritis, nervous dyspepsia, phthisis, cardiac disease; especially in (1) severe febrile diseases, particularly the infections; (2) gastric carcinoma (also other carcinomata); (3) atrophic gastric catarrh; (4) pernicious anæmia. The most important is the failure of free hydrochloric acid in gastric carcinoma.

And yet gastric analysis is not pure mathematics; the figures are simply relative to the case, some patients being distressed by an acidity normal to another man. On the other hand, in all cases the absence of free hydrochloric acid is abnormal.

Standards vary with nationalities, but especially with classes of society and still more with individuals. Strauss, at Giessen, thought 68 a fair average total acidity; at Berlin, 47. In this country we must not try to make German acidities fit any more than the meals producing them; and if possible, every case should be considered individually.

Among 526 cases of this clinic whose records were studied are the following. In all cases the Ewald breakfast was used.

Pernicious Anæmia, 13 cases. Amount removed, 10 to 80 cc. All were subacid, the highest total acidity being 38 (acidity per cent.) and below 10 in 10 cases. In only one was any free hydrochloric present. In two the fluid was neutral to litmus; in one alkaline. Lactic acid was present in two cases (in one there was an autopsy, in the other not). In two cases of severe *secondary anæmia* the fluid was only slightly subacid, and free hydrochloric acid was present.

Malignant Disease not of the Stomach.—Of these, ten were carcinomata, four sarcomata. All were subacid (total acidity less than 40). Of the carcinoma cases, free hydrochloric was present in seven, absent in two, and the fluid neutral to litmus in one. Of the sarcoma cases, in none of the four was free hydrochloric present.

Catarrhal Jaundice, 9 cases. The fluid removed varied from 10 to 86 cc.; total acidity, 10 to 70; in three cases no free hydrochloric acid; in one the fluid was alkaline to litmus; in none was lactic acid found. In a few cases the acidity progressively diminished during the course of the disease.

Cholelithiasis, 14 cases. Amount removed 5 to 120 cc. There was hyperacidity in one case (total 79 and 82; free HCl, 42 and 49 respectively on two examinations). Normal acidity (40 to 70) in six cases; below 40 in six; and in four of these no free hydrochloric; lactic acid in one.

Cirrhosis of Liver, 6 cases. Normal acidity obtained in one, subacidity in five,

in four of which was no free hydrochloric acid, and one was practically neutral, in one, lactic acid.

Tuberculosis of Lungs, 10 cases. Normal total acidity in three; subacidity in seven; no free acid in two. Tuberculosis of other organs, five cases; normal acidity in one; no free acid in three; in one of these the fluid was neutral; in one lactic acid present.

Intestinal Troubles.—*Diarrhœa,* 9 cases; in four the acidity was normal; in four subacid, in one almost neutral. In four no free acid was present; in one was lactic acid. *Constipation,* 5 cases, of which two were normal, three subacid, one without free hydrochloric. Colitis, 2, both subacid and without free acid, and both with lactic acid. Amœbic dysentery, one hyperacid (92 total and 83 free).

Arteriosclerosis and Cardiac Diseases, 17 cases. Eight normal, six subnormal, three without free acid, and one almost neutral.

In a group of 36 cases of Miscellaneous diseases, twenty-five showed normal gastric conditions. Subacidity without free acid was present in cases of heart prostration, enteroptosis, chronic bronchitis, peripheral neuritis (with lactic acid), chronic nephritis, bronchopneumonia, and malaria.

Various other methods of *testing the gastric juice without using a tube* have been proposed. (The reader is referred to Herschell, "Manual of Intragastric Technique," 1903.)

Dunham advised a thread with a tassel on the end soaked in a reagent (as dimethylamidobenzol), which is easily swallowed, the thread passing through a glass tube through which the patient drinks water, and again easily withdrawn and the color noted.

Turck's capsule consists of a rubber tube attached to a silk thread, and having attached to it various reagent papers. It is enclosed in a gelatin capsule. This is swallowed one hour after an Ewald breakfast, withdrawn in fifteen minutes, the papers inspected, and a few drops of juice in the rubber tube tested and examined microscopically.

Einhorn's stomach bucket is a small silver bucket holding about 2 cc., attached to a strong silk, which is easily swallowed and withdrawn.

Pepsin.—We do not think it suitable here to consider the question of the nature of pepsin, pseudo-pepsin, etc. The qualitative determination of pepsin is less important than that of the hydrochloric acid, since pepsin is practically always present when there is any and usually when there is no free hydrochloric acid; that is, the pepsin-forming function of the stomach seems more resistant than the hydrochloric acid function, and the water secreting function most resistant of all. Again, if the gastric juice contains hydrochloric acid, the appearance of the contents would indicate at once if pepsin were also present. Its determination is, therefore, limited to those cases without the free acid,—carcinoma and atrophic gastric catarrh. Schiff found that in hypoacidity and anacidity the pepsin does not change much, but that there is a considerable diminution in cancer, even when that of the acid is only slight, hence he considers it important in the early diagnosis of carcinoma. It is absent in atrophic catarrh, gastric carcinoma, and

certain cases of pernicious anæmia. Its absence is always a bad prognostic sign of recovery.

Qualitative Determination.—The presence of pepsin is assumed if the acid gastric juice (HCl added if none be free) will digest egg albumin or fibrin. The fibrin is prepared as follows: Fresh ox-blood is whipped and the fibrin kept in running water until perfectly colorless. It is then cut in fragments of equal size, put for a few days in alcohol, and then for one or two days in cool concentrated neutral carmine solution until fully stained. It is then well washed and pressed and kept in glycerin stained with carmine. Before use the fibrin is well washed with water to remove all glycerin. For egg albumin, the egg is boiled until perfectly coagulated (about five minutes). Long boiling should be avoided, since the albumin is made more difficult to digest. The white of the egg is then cut into 5 mm. cylinders, using an ordinary cork borer, and these into disks 1 mm. thick. These disks are kept in glycerin, and also are washed in distilled water before using (Sahli).

To gastric juice is added hydrochloric acid, if necessary, until the Congo-red paper shows free acid, then a few fibrin or egg fragments, and the whole put in a thermostat. If pepsin is present the fibrin will show signs of digestion in from fifteen to thirty minutes, the egg albumin in one-half to four hours. If fibrin is used the liberation of the carmine is a very early sign of beginning digestion. The first sign for the egg albumin disks is the rounding of the edges. Riegel prefers egg albumin to fibrin, since one gets more constant results as regards time and it is easier to control the mass used. Sahli recommends that both be tried since some gastric juices can digest the fibrin easily but not the albumin. For a perfect test he recommends that five tubes should be prepared for fibrin and five for albumin;

Tube 1: 5 cc. gastric juice plus carmine fibrin.

Tube 2: 4.5 cc. gastric juice plus 0.5 cc. 2 per cent. HCl and the fibrin.

Tube 3: 5 cc. gastric juice plus 0.05 gm. pepsin and the fibrin.

Tube 4: 4.5 cc. gastric juice plus 0.5 cc. 2 per cent. HCl, pepsin and the fibrin.

Tube 5: 5 cc. gastric juice plus 5 cc. water.

Tubes 6 to 10: The same series but using egg albumin.

Sahli recommends that these tubes be left at room temperature, that the difference in them may be more clearly observed. If in tubes 1 and 6 digestion is present in a half to three-quarters of an hour pepsin is normal and the hydrochloric acid normal or increased. The same is true if tubes 2 and 3 are not better than 1. Only rarely does one get complete digestion without free acid and then it is probable that lactic acid acts in its place. But in this case tube 2 will show better digestion.

Very rarely tube 3 is the best; usually tube 4 is when any pathological condition exists. When dilution improves the digestion (and then 5 is best) it means that motility is deficient.

Quantitative Determination.—The general law of ferment action is that its activity (as shown by the products of its digestion) varies as the square root of the amount of the ferment. The truth of this formula has been often tested. Caudet determined the nitrogen of the coagulable and uncoagulable proteid by the Kjeldahl method. Schütz and Huppert as the result of very careful work consider the formula $s = ka\sqrt{p.t.s.}$ to hold ($k =$ constant; $s =$ deuteroalbumose; $a =$ albumin used; $p =$ pepsin; $t =$ time; $s =$ amount of HCl).

Hammerschlag used a 1 per cent. albumin solution with 0.4 per cent. free HCl which he put in two tubes, 10 cc. in each. To the one he added 5 cc. of water, to the other 5 cc. of gastric juice. These were left in a thermostat one hour and the coagulable albumin determined by Esbach's tube. The errors of this method are, those of the Esbach method, that the albumin of the gastric juice itself is not inconsiderable, and that one hour is too short a time. Normally, 80 to 95 per cent. of the albumin is digested.

The method of Mett is at present that most used. Egg albumin is filtered and the gas removed by a suction pump for several hours. A beaker is filled with the albumin, a bundle of glass tubes, each about 10 cm. long and 1 to 2 mm. wide, are then filled with the albumin by immersing them in it. Beaker and all are then put for five minutes in boiling water at 95° C. The tubes are then carefully removed, their outside cleaned, and both ends closed with sealing-wax. In the former method of filling the tubes by aspiration and then holding the tube in boiling water, it was found that the tubes were not so well filled, although on allowing them to stand the bubbles would disappear. When ready for use the tubes are cut in pieces from 1 to 2 cm. long and placed in the fluid to be tested and this in a thermostat. A very small amount will suffice, in which case it is better to use a watch-glass. It is left ten or twenty-four hours in a thermostat. At the end of that time, under the low power of a microscope, the length of the digested column at the ends is measured and their average estimated. This test has been used by many,[6] and the conclusions were that pepsin varied as much as HCl. But it was soon found that the digestive power was decreased in the presence of sodium chloride or carbohydrates, or the products of proteid digestion. (This inhibition of the ferment is best seen in cancer and chronic catarrhal gastritis.) Hence Nierenstein and Schiff advised to always dilute the fluid sixteen times, that pepsin in this dilution may obey the formula. One cc. of the filtered gastric juice is mixed with 15 cc. of twentieth-normal

[6] *E.g.*, Roth, Zeitschr. f. klin. Med., Bd. 39, p. 1.

HCl and well shaken. Into it are dropped the fragments of the glass tubes and allowed to remain for twenty-four hours in the thermostat. The square of the average length multiplied by 16 will give the units of pepsin. (By unit is meant the amount of pure pepsin which in twenty-four hours will digest an average of 1 mm. of the albumin in the tubes.) In these dilutions they found the length of the column digested varied from 0 to 4 mm. The length which theoretically pure pepsin would give is 4 mm., yet the undiluted gastric juice often gave from 4 to 6 and even 8.6 mm., hence the law hardly holds for the normal gastric juice, the amount of pepsin of which should be from 0 to 256 units. In gastric juice with very little pepsin the fluid diluted sixteen times gives no digestion, hence they recommend that a preliminary test be made with fibrin to determine the necessary dilution.

Volhardt's method is the one which Riegel prefers. He digests an HCl-casein solution by the gastric juice. The casein is then precipitated by Na_2SO_4 and the filtrate titrated. The total acidity will be higher the more the casein is in uncoagulated form and the increase in acidity will vary as the square root of the pepsin. By this method the least trace of pepsin can be determined.

The Fat-Splitting Ferment.—The presence of this was emphasized by Volhardt.[7] To demonstrate it the yolk of one egg is mixed with 30 to 40 cc. of water; 10 cc. of this mixture is mixed with the gastric juice, both of which fluids have been warmed separately in the thermostat, and then the mixture placed in a thermostat at from 37° to 40° C., then cooled. Seventy-five cc. of ether and a few cubic centimetres of alcohol are then added and the whole shaken out. A measured amount of this fat-containing ether is mixed with 50 cc. of neutral alcohol and titrated with tenth-normal NaOH, phenolphthalein as indicator. The result is the fatty acid. To the titrated mixture are now added 10 cc. of tenth-normal NaOH and placed on a water-bath for two hours. The flask is connected with a condenser and a calcium oxide tube to exclude CO_2, or is allowed to stand for twenty-four hours in a closed flask at room temperature. This is to saponify the unsplit fat. Ten cc. of tenth-normal HCl are now added to free the fatty acid and the mixture again titrated with tenth-normal NaOH, phenolphthalein as indicator, to determine the fat which had been unchanged in the thermostat. From the relation of these the percentage of split fat is reckoned, and hence the number of units of ferment present. This ferment is very sensitive to alkali. It has been shown that its activity varies as the square root of the amount of the enzyme. Stade's formula is: $p = \sqrt{f} \times \sqrt{t}$ in which p equals the products of digestion, f the units of ferment, and t the time. If f represent the

[7] Münch. Med. Wochenschr., 1900, v. and vi.; Zeitschr. f. klin. Med., Bd. 42 and 43.

amount of ferment which will digest 1 per cent. in one hour, then if in three hours 6 per cent. of the fat be found split, twelve units of ferment were present.

It has been found that in hypochylia and achylia this ferment is absent or diminished. Riegel considers that much of the former work which has been spent on hydrochloric acid should now be spent on ferment action. It is needless to say that the demonstration of this fat-splitting ferment throws considerable disrepute on those test meals of neutral fats which have been proposed. Volhardt considers that in two hours from 30 to 36 per cent. of the fat is split. These fatty acids dissolve in the bile and aid in the emulsion of the neutral fat. Hence the stomach really does considerable of the work of the pancreas.

Rennet.—The presence of rennet is proved by the coagulation of milk by the neutralized gastric juice. Leo's method is as follows: From 5 to 10 cc. of milk and 3 to 5 drops of gastric juice are placed in the thermostat. A better method (Riegel) is the mixture of from 5 to 10 cc. of gastric juice neutralized with tenth-normal NaOH and 5 to 10 cc. of fresh milk. Normally, this will coagulate in from ten to fifteen minutes. If slower, lactic acid formation must be excluded. The reaction is unchanged by the ferment. The presence of the zymogen in the absence of the ferment has been much disputed. As a rule, this ferment varies with pepsin, but it is easier to test for.

Boas attempted quantitative determination by mixing variously diluted neutralized gastric juice with an equal amount of milk until no coagulation occurred. Normally this would cease at a dilution of from 1 : 100 to 150; in severe cases, however, when the dilution was only from 1 : 5 to 10.

The practical importance of the quantitative determination of this ferment is uncertain, since the methods are not good enough. Glassner considers that it has diagnostic value; for instance, that in pyloric cancer this ferment is uninjured. If the pepsin and the rennet are both much diminished he considers we have a tumor of the fundus; if the pepsin is diminished and the rennet good, one of the pylorus.

The extent of gastric digestion is considered by some important. J. Müller fed a patient from 1 to 200 gms. of finely ground meat and found that in one hour 28 per cent. of the albumin was dissolved. This is a minimal amount, since some has passed to the intestine. In supersecretion and hyperacidity the albumin digestion was much increased, especially if at the same time there was pyloric stenosis. But in subacidity, chronic gastritis, cancer of the stomach, it was much diminished.

Benedict [8] has given a simple method of determining the products of digestion. The albumin is precipitated by heat, the albumoses by

[8] Am. Jour. Med. Sci., 1904, vol. cxxvii.

ammonium sulphate and the peptone by phosphotungstic acid. The various precipitates are determined volumetrically by centrifugalizing them to the smallest volume.

The Products of Albumin Digestion.—One may determine this if he will, and yet from the practical point of view it is of little importance. From a theoretical point of view it is interesting to note that in cases of carcinoma of the stomach digestion of proteid is so much more rapid than normally that it is safe to conclude that an abnormal ferment is present.[9] This is rendered very probable, since artificial digestion experiments with the heated and unheated carcinoma tissue show a similar relation.

In our benign cases tested in this way, the Ewald breakfast being used, the average amount of nitrogen in albumose form was 51.7 per cent.; in the phosphotungstic acid precipitate, 31.4 per cent.; in the residue, 16.9 per cent. In the carcinoma cases these figures were respectively 27.5, 47, and 27.6 per cent.

Starch Digestion.—It has recently been again emphasized [10] that the saliva is not as unimportant in starch digestion as was formerly supposed, and that it renders soluble in the stomach from 50 to 70 per cent. of the starch, thus relegating the starch digesting function of the pancreas to a subordinate position. This digestion occurs, however, before total hydrochloric acid has reached 0.12 per cent., hence is inhibited in cases of hypersecretion and hyperacidity. Concentrated lactic acid also can inhibit this process.

The stages of starch digestion are soluble starch, erythrodextrin, achroodextrin, and maltose. The relative amount of this may be approximately detected by the use of a very weak Lugol solution, since the later products of starch digestion have a greater affinity for the iodine than have the earlier. The colors obtained vary from blue to colorless, the first blue-violet, the erythrodextrin red to mahogany-brown. One drop of the weak Lugol's added to a small amount of gastric juice will therefore give no blue color with starch if much achroodextrin be present. On the other hand, if very few of the higher products be present, it will give a distinct blue color. From the number of drops to be added before the blue color appears may therefore be approximately determined the relative extent of the starch digestion. This has been considered a good indication of the amount of free acid, since in cases with high acidity the first drop may give the end reaction. And yet it should be remembered that the saliva is also to be taken into consideration; that of smokers, for instance, is supposed to have less digestive power than normal.

Lactic Acid.—The test for lactic acid is of value only when it is known that the meal contained none, and that if any is formed from any constituent of the meal it would normally have disappeared at the time of the test. Riegel says its presence in the stomach is never normal except during sugar digestion. One usually tests the juice

[9] Deutsch. Arch. f. klin. Med., vol. lxxii. p. 415.
[10] J. Müller, Verh. d. XIX. Congr. f. inn. Med.

after an Ewald breakfast, but this is hardly fair (see page 349). Dock uses a shredded wheat biscuit.

Some breads contain lactic acid, hence Boas proposes a meal of one tablespoonful of oatmeal cooked in one litre of water with a little salt. The stomach is well washed out and this meal given in the evening and removed the following morning. It is said that normal saliva can produce lactic acid from this meal. This meal is little used.

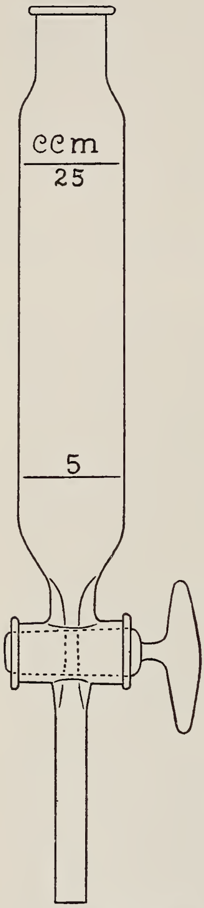


Fig. 63.—Strauss' separating funnel for lactic acid tests.

Uffelmann's test is the one in common use. This solution is always made up fresh. To about 20 cc. of 1 per cent. carbolic acid in a test-tube is added one drop of 10 per cent. Fe_2Cl_6. A deep amethyst color is produced. This is diluted with distilled water until the fluid is fairly transparent. It is then halved. To the one test-tube is added a drop or so of the gastric juice, to another the same amount of distilled water. If lactic acid be present, in the former the fluid takes a definite yellow or yellowish-green (canary-green) color.

Others propose 10 cc. of a 4 per cent. carbolic acid in 20 cc. of distilled water and one drop of the ferric chloride. Others one drop of the ferric chloride solution in distilled water until almost colorless, and that the carbolic acid (2 to 4 per cent.) added until the proper color is produced.

It should be noted that decolorization alone is not sufficient. A definite canary color or yellow is necessary. The blue is merely for contrast, hence one may dispense with the carbolic acid. It is well to control the test with dilute lactic acid.

The test as given by Strauss is particularly valuable. In a small separating funnel (see Fig. 63) with two marks, one indicating 5 and the other 25 cc., are introduced 5 cc. of the gastric juice. The funnel is then filled to the 25 cc. mark with alcohol-free ether and well shaken to extract the lactic acid. One drop of hydrochloric acid may be added to liberate that lactic acid which is bound to proteid. The gastric juice is then allowed to run out and 5 cc. of distilled water added, then 2 drops (from a medicine dropper to insure uniform size) of a 1 per cent. Fe_2Cl_6 solution. If 0.1 per cent. of lactic acid be present, the water layer will take a definite canary-green color. This test is perhaps not as delicate as some others, but it may be said confidently that if positive a pathological amount of lactic acid is present. Always to extract with ether is quite necessary,

since simple decolorization or even a suspicious color may be given by sugar, proteid, and alcohol, and by some other inorganic acids (oxalic, citric, tartaric), and positive tests may be prevented if much phosphates or peptones are present. Again, ferric chloride may give a cloud in the gastric juice which will obscure the test. If, however, the result of an Ewald breakfast given on a clean stomach be examined, trouble from these sources will not occur.

This test has been further modified. Kelling [11] dilutes the fluid with twenty volumes of water and then adds one or two drops of 5 per cent. Fe_2Cl_6. The tube is then sighted by transmitted light, and it is stated that from it 1 of lactic acid in 10,000 to 15,000 of water can be detected. Knapp [12] adds 1 cc. of gastric juice to 5 cc. of ether and shakes. He then superimposes this extract upon a colorless ferric chloride solution (1 : 2000) freshly prepared, and gets a canary-colored ring.

De Jong [13] adds to 5 cc. of the gastric juice one or two drops of HCl. He evaporates this to a syrup over a free flame and then extracts the residue with a little ether. The volume is then made up to 5 cc. with distilled water and one drop of 5 per cent. Fe_2Cl_6 added, and the whole well shaken. A definite green color is produced by 0.05 per cent. lactic acid.

Quantitative Lactic Acid.—For clinical purposes the Strauss modification of the Uffelmann's method is accurate enough, and perhaps of greater value than the more accurate methods, which might show small amounts normally present. It is only the pathological amounts that are of value. The Hehner-Maly method is satisfactory if one consents to consider all organic acids as lactic.

Some idea of the amount may be obtained by comparing the acidity of the gastric juice before and after extracting it with ether.

Boas Method.—The principle of this method is to oxidize lactic acid to aldehyde and formic acid. The aldehyde is then changed to iodoform and this determined.

Either 10 or 20 cc. of the filtered gastric juice in a porcelain dish are evaporated to a syrup on the water-bath. If free HCl be present, $BaCO_3$ is added in excess to prevent its volatilization. To the syrup a few drops of phosphoric acid are then added to again set the acid free and then the syrup heated to drive off the CO_2. It is then cooled and extracted two or three times with 50 cc. of ether. After standing about half an hour the ether is poured off, evaporated to a residue, this taken up with 45 cc. of water in a flask, then shaken and filtered. To the filtrate are added 5 cc. of concentrated H_2SO_4 (sp. gr. 1.84) and a knife-point of manganese. This flask is then fitted to a distilling apparatus with two tubes through its cork, the one for the cooler and the other for the clamped rubber tube through which air may later be blown in order to cleanse the cooling tube from the aldehyde, and the fluid distilled until about four-fifths has passed over. The other end of the cooling tube dips into a well-closed Erlenmeyer flask containing 10 to 20 cc. of tenth-normal iodine solution in 20 cc. of KOH. This is shaken, closed tightly, and allowed to stand for a few minutes. The aldehyde will be changed to iodoform. To it are then added 20 cc. of HCl (sp. gr. 1.018) and an excess of sodium bicarbonate. It is then titrated with a tenth-normal sodium arsenite or tenth-normal thiosulphate solution, which has been accurately

[11] Zeitschr. f. physiol. Chemie, 1903.
[12] New York Med. Jour., August, 1901.
[13] Arch. f. Verdauungskranheiten, Bd. 2.

standardized to the tenth-normal iodine solution, until decolorized. A little fresh starch solution is then added, and one titrates back to the first permanent blue. The amount of iodine solution less that of the tenth-normal thiosulphate solution gives the amount of the former necessary to form the iodoform. One cubic centimetre of the iodine solution equals 0.003388 gm. of lactic acid. Or the iodoform may be estimated gravimetrically (see page 186).

Other Organic Acids.—Acetic, butyric, and valeric acids occur and are recognized by their odor. They are the result of bacterial decomposition, although the reason why in some cases one, in some another, is formed is not known. For instance, in a recent case the patient after an Ewald test meal complained of the taste of vinegar and the gastric contents were found rich in acetic acid. This man had given all the symptoms of hyperacidity.

Much organic acid could come from the foods, from fermentative processes in the stomach, or from the action of the fat-splitting ferment.

On a lactic acid-free meal, and only such should be used, one should normally find no lactic acid in the stomach. Lactic-acid-producing bacteria are in the mouth, but should not have enough time to produce the acid in the stomach.

Acetic acid carefully neutralized with soda is tested with 1 or 2 drops of Fe_2Cl_6 solution, which gives the bluish-red color of ferric acetate.

Butyric acid in the presence of fine fragments of $CaCl_2$ separates in fine oily droplets.

Total Organic Acid. *Hehner-Maly Method.*—The principle of this method, which has been recommended by many, is that if a mixture of organic and inorganic acids be ashed the inorganic salts will remain neutral, while the organic will be changed to alkaline-reacting carbonates. The original acidity minus the final alkalinity may be considered to equal the mineral acidity.

Ten cc. of filtered gastric juice are neutralized with tenth-normal NaOH, using phenolphthalein as an indicator (number necessary $= a$). This fluid is then evaporated and ashed, the ash taken up with water and titrated with tenth-normal HCl, phenolphthalein again used as indicator (amount necessary $= b$) $a - b =$ the mineral acidity.

The method is easy. By it the acid phosphates are included with HCl, hence in its original purpose as a method of determining HCl the phosphates must be separately determined; but it is the easiest method to determine the organic acids.

Bases of the Gastric Juice.—Of these sodium is the most important. V. Mehring has shown that the stomach can and does secrete sodium carbonate to control the amount of hydrochloric acid. Reissner showed an increased secretion of alkali in cancer.

A base present in no small amount is ammonia (normally 0.1 to

0.15 p. M.). The gastric wall is the tissue of the body richest in this base. This amount of ammonia in the gastric contents throws great doubt on the value of the titration with phenolphthalein as indicator. The source of the ammonia is doubtful, some even say all is from the saliva.

Fermentation.—Fermentation with gas formation occurs in dilatation of the stomach even in the presence of free HCl in case the stasis be sufficient, never when motility is normal; more rarely in diminished or an-acidity, in which case lactic acid formation is more common. The fresh gastric juice or vomitus unfiltered is well shaken, and poured into two fermentation tubes, the second as a control to which is added a small amount of glucose, since all of the carbohydrate may have already been fermented. If there is no gas in twenty-four hours it is advised to wait from three to four days. The chemical analysis of the gas is not very important. This test is important in determining the degree of stagnation, although fermentation without gas production does occur, and there is a small amount of gas formed in the normal stomach when much food is ingested, or when there are clefts or pockets in the stomach wall.

A great many different organisms have been isolated and studied,[14] among them yeasts and sarcinæ in those cases with free acid, and long bacilli in cases of cancer. Sarcinæ are usually present when there is high acidity. "If there be slight total acidity and sarcinæ the evidence is for cancer. High total acidity with short bacilli speaks against cancer; while low total acidity with many long bacilli, in favor. As products of such fermentation may be mentioned alcohol, methane, ethylene. Sarcinæ alone have been found to produce ethyl alcohol, aldehyde, acetic and formic acid, but not lactic nor butyric, nor carbon dioxide nor hydrogen. Coyon considered that the sarcinæ have little fermenting power, and isolated two bacilli,—the enterococcus, which produces lactic acid, acetic acid, and ammonia bodies from proteid, lactic and acetic acids from carbohydrates; and Coccus radiaire, which from albumin builds fatty acids. Yeasts are common, producing carbon dioxide, alcohol, aldehyde, and acetic acid from carbohydrates, and hydrogen disulphide and ammonia compounds from albumin (Groot).

Hydrogen disulphide from decomposing proteid is rare in cancer but quite common in benign gastric dilatation, and may be detected by its odor or by suspending over the fluid a paper wet with alkaline sugar of lead. Dauber found in nearly every stomach bacteria which can produce this gas from sulphur bodies, and, in fact, given long enough time, nearly all bacteria can do this, hence there is nothing specific in its presence in the stomach. But it cannot be produced if there is free hydrochloric acid present. On the other hand, that from

[14] See Ehret, Mittheil. aus der Grenzgeb. d. Med. u. Chir., Bd. iii. Heft 5.

the food must always be excluded; for instance, from radishes, onions, and also from the saliva.

Microscopical Examination.—This is seldom of much value since a large amount of unchanged starch granules and muscle-fibres pass to the intestine. In cases with poor gastric secretion the muscle-fibres are particularly well preserved with their cross striation, but if there is much stasis even this is no sign. Mucus may be recognized from its fibrillar character and the cells that are embedded in it, also by staining it with safranin or Ehrlich's triple stain.

Pus is very rarely found macroscopically. It might be expected in gastritis phlegmonosa, in abscess of the stomach, and in rupture of one through the stomach, especially a subphrenic abscess. Strauss [15] has also found it in a case of cancer of the fundus and lesser curvature, with subphrenic abscess, in amounts from 60 to 500 cc. Large numbers of pus-cells may be found in cancer of the stomach, and we had one case in which much was present microscopically, yet its diagnostic value is to be doubted. Microscopically, a few pus-cells are always found in the wash-water of an empty normal stomach. The fasting stomach should be examined, since when mixed with food the cells cannot be recognized; also it is seen best in stomachs with good motility.

Fragments of mucosa are often torn loose during lavage without any bad result following. This is particularly true if suction with a pump or Politzer bulb be made. Yet if the tube be carefully used their presence may in a general way indicate the vulnerability of the mucosa. Some of these fragments from the proliferating mucosa, in cases of achylia gastrica, will give a very good picture of carcinoma. The morning lavage of the stomach may show many of these fragments, and then is the time when it is suggested to search for fragments of tumors. In a reported case of Heynoch's purpura such fragments of mucosa were found in the washings; the wall of the stomach was quite œdematous (Dr. Morris). In the fasting stomach also may be found characteristically shaped groups of nuclei of leucocytes whose protoplasm has been digested. In the fasting stomach also may be seen myelin masses, altered by HCl, which have the appearance of snail-like spirals. Crystals are also present, especially if bile be mixed, of cholesterin, leucin, calcium oxalate, and triple phosphate. In conclusion, the microscopical examination is interesting rather than important. Of greatest importance are fragments of carcinoma. Many yeasts, especially if they occur in chains, sarcinæ, and long bacilli, are also important.

Infusoria are sometimes present (see page 375) in the gastric juice, the reaction of which must be neutral or alkaline.

[15] Berl. klin. Wochenschr., 1899, No. 40.

The greatest variety of BACILLI are of course found. EPITHELIAL CELLS occur often in abundance, particularly when digestion is poor. CANCER FRAGMENTS are very rare. If free hydrochloric acid be present, MOULDS and YEASTS and SARCINÆ will predominate; if none, BACTERIA. Einhorn found in the wash-water spores of moulds which he thought lodged in crevices of the mucosa and might aid in the hyperacidity and the gastralgia. Yeasts are abundant in gastric dilatation, and a few may be found in normal stomachs. Sarcinæ occur in large numbers in benign dilatation, occasionally in gastritis, ulcer, and neuroses, but rarely in cancer. One observer reports them in such numbers as to form plugs obstructing the pylorus. Ehret found many sarcinæ in cases of intense fermentation, but neither yeasts nor bacteria, and considers that they indicate a severe stasis. He does not consider that they occur only with free hydrochloric acid. Two sizes may be found, the large and the small. In a recent case in this clinic with dilated stomach the sarcinæ were in great numbers, and of huge size.

BLOOD.—The œsophagus, nose, mouth, and lung as a source must be excluded. Small amounts of blood have no significance, since slight lesions of the pharynx, œsophagus, or stomach are very easily produced in vomiting or by the tube. Blood may be present in large amounts in ulcer of the stomach, or rupture of venous dilatations at the cardiac orifice as in liver disease. In case the stomach be empty, the blood will then be arterial, but, as a rule, it is dark because of the hæmatin produced by the gastric juice, and is clotted and well mixed with food. In cases of carcinoma of the stomach there is usually a slight constant bleeding, the blood then well mixed with the food and so digested that it gives a coffee-ground appearance. This is a very valuable sign in cases of gastric dilatation, indicating cancer of the mucosa. It also occurs in hyperacidity with hæmorrhagic erosions of the mucosa. The detection of this small amount of blood is no easy matter, since microscopically nothing is found. Blood may arise from tuberculous ulcers; slight injuries to the mucosa; from small aneurisms of the gastric arteries due to trauma; the digestion of the mucosa over an infarcted area; in chronic passive congestion; cirrhosis of the liver; various constitutional diseases without apparent reason, as in anæmias, hæmophilia, Hodgkin's disease; active hyperæmia as in vicarious menstruation; and following abdominal operations, especially such as involve the omentum, in which case they are a disturbing symptom, but of no moment.

All the blood of even a profuse hemorrhage may pass by the stools.

Occult hemorrhage discovered by examining the gastric contents and stools occurs in several conditions. Boas and Kochmann[16] divide cases as follows: Those with no hemorrhage; gastritis anacida, sub-

[16] Arch. f. Verdauungsk., 1902, vol. viii. Heft 1, 2.

acidity, hypersecretion, benign ectasis; cases with blood at times, especially ulcer; cases with blood as a rule, especially cancer.

Deen's Test.—To the gastric juice is added 1 cc. of fresh tincture of guaiac and 1 cc. of Hühnerfeld's solution (glacial acetic acid, 2; distilled water, 1; oil of turpentine and alcohol, of each, 100 cc.). The mixture is well shaken. If blood is present the fluid turns blue. The test is also given if iron compounds are present, or some vegetables, etc., hence it has chiefly a negative value. Weber's modification is recommended by Riegel: to the gastric contents is added one-third volume of glacial acetic acid and it then shaken out with ether. After the ether extract has cleared, to a few cubic centimetres are added 10 drops of guaiac tincture and 20 to 30 drops of turpentine. Blood will give a bluish-violet color. In this case only raw or rare-cooked meat is to be excluded.

Heller's test is also used, but various red substances must be excluded, coffee, cocoa, wines, etc. The precipitate should be collected on a filter and dissolved in acetic acid. Rhubarb, senna, and santonin may deceive.

For the spectroscopic test the gastric contents are diluted with water, a few drops of concentrated acetic acid added, and then shaken out with one-fifth volume of ether. In a few minutes a clear layer of brown ether solution of hæmatin is obtained. The four-band spectrum of hæmatin in acetic acid could be due to chlorophyll, hence alcoholic KOH is added and it reduced with $(NH_4)_2S$, the red fluid will now give the two-line spectrum of reduced hæmatin.

Absorption Power of the Stomach.—Although this function of the stomach may not be important, yet there are certain substances which are absorbed, as alcohol, sugar, dextrin, peptone, albumoses, *et al.,* and in amounts varying with the quantity in the stomach. The absorption test generally applied is that of Penzoldt. On an empty stomach is given a gelatin capsule containing 0.2 gm. of potassium iodide. The saliva and urine are examined every few minutes until the test is positive. To the saliva or the urine are added a little starch meal and fuming HCl. The blue color will indicate the presence of iodine. Or a little starch meal is added and crude HNO_3, but an excess of acid must be avoided or the first trace of iodine will be lost. Also the sputum and starch must not be left in contact long as the digestion will give erythrodextrin and achroodextrin. Chloroform may be used instead of starch, and will take up the iodine to a pink solution. Normally the excretion begins in six and one-half to fifteen minutes in the sputum, and in the urine in thirteen and one-half minutes. The time is shortest on an empty stomach and at the height of digestion. The test should be used under constant conditions, hence Sahli combines it with the Ewald breakfast, in which case

iodine appears in five to twenty-five minutes. There is a delay in most gastric diseases. The test has chiefly a theoretical, very little practical value. Only a considerable delay counts. This may occur in dilatation with considerable catarrh, and carcinoma; there is no delay in ulcer without catarrh, nor in neurotic disturbances. Potassium iodide is not food, and it is not absorbed from a stomach which is closed off from the intestine, hence the test may show when it first reaches the intestine.

Again, of importance is the SECRETION OF WATER, which is the most resistant function of the gastric mucosa and may be stimulated independently of the other secretory functions. It is increased owing to the presence and absorption of alcohol, sugars, etc. This "dilution secretion" is supposed to be a measure to protect the intestine, the increased fluid dilating the gastric contents.

Motility of the Stomach.—It is to be emphasized that disturbance of the motility of the stomach is far more important than disturbance of its secretions. If motility be good the intestine can vicariously make up easily for any insufficiency on the part of gastric secretion, and the person live years in ignorance of the fact that he has no gastric juice. But if motility be impaired the stagnation of food in the dilated stomach soon and always produces serious results.

Hypermotility is seen especially in cases of hyperacidity, and it may be necessary to remove the Ewald breakfast in from one-half to three-quarters of an hour in order to obtain any fluid. But the most rapid motility is seen in cases of jejunal fistula high up. In these cases of starvation the stomach seems to try to hurry the food into the intestine at as rapid a rate as possible. In one such case a glass of milk was drunk and collected at the fistula. It appeared in one minute, and was wholly recovered in four minutes from the time it was swallowed. The surgeons used the following method of determining the position of the fistula. An oyster was tied with a piece of silk thread and swallowed. In a few minutes it appeared at the abdominal opening. The thread was then cut at the teeth, pulled through and measured.

It is quite important to wash the stomach out to be sure it is empty, since in cases of achylia the tube may siphon nothing, but the wash-water may show considerable solid matter.

Megalogastria means enlargement of the stomach, and may or not be accompanied by motor insufficiency.

Ectasis refers to enlargement with motor insufficiency; it is "atony" if due to real weakness of the muscle wall, is "hypertonic ectasis" when due to pyloric stenosis.

Motor insufficiency may be absolute or relative and dilatation of the stomach is in general due to one of two factors,—(1) atony of the gastric wall, in which case the muscle is not strong enough to

empty the stomach; in this group are found the largest stomachs. Strauss reports a case[17] of five and a half liters capacity. (2) Muscular insufficiency, that is, an obstruction at the pylorus which renders exit of the food difficult. In such a case the muscle wall may be abnormally strong and hypertrophied; in others there is no dilatation of the stomach, since the wall is extensively infiltrated.

Stenosis at the pylorus causing dilatation may be congenital or acquired. In the latter group are the contractions of scars of ulcers; the result of swallowed irritants; cancer; or the hypertrophic stenosis explained as due to the continual cramp at the pylorus from irritation by hyperacid juice or an ulcer; and the pressure of external tumors; adhesions, kinks, twists, diverticula.

In a normal case, no matter how large the meal, the stomach should be empty in seven hours. A common test of motility is to give without previous lavage a simple evening meal but one of constant composition, as of cold meat, bread and butter, and tea (Boas). If the following morning food be found, there is considerable motor insufficiency. If before this evening meal the stomach had been well washed out and found empty in the morning, the degree of insufficiency is less; if food is found, even more. If the stomach contains food seven hours after a full noon or morning meal, but none after a night's rest, the degree of insufficiency is least.

Ewald and Strauss have recommended to give one spoonful of currant or raisin preserve with the evening meal. The seeds of this can be recognized in the stomach washings the next morning, no matter if the patient has taken a large breakfast.

If in the morning the fasting stomach contain over 100 cc. of fluid, motor insufficiency may be suspected and the increased secretion attributed perhaps to the stimulus of some fluid or food which remains, but such a stomach will be empty if the evening before it had been washed clean.

The symptoms of dilatation are those of the disease causing it, and the vomiting of large amounts of food which has been eaten more than seven hours, in some cases even three days, before. The vomiting of food in the morning before breakfast is a sure sign.

To please those patients who object strenuously to a stomach-tube the following method of Ewald and Sievers is used. It is based on the belief that salol remains unchanged in the stomach, but is split by the pancreatic juice and bacteria to salicylic acid and phenol. The salicylic acid is excreted in the urine as salicyluric acid, which may be easily detected by the violet color on the addition of ferric chloride to the urine. The test assumes that the time of splitting, absorption, and excretion remains constant, which is not always true. One gramme of salol is given with the test breakfast and the urine is examined at intervals. The test should appear in the urine at the latest in seventy-five minutes.

[17] Deutsch. med. Wochenschr., 1904, No. 15.

If the first appearance is delayed longer there is certainly motor insufficiency. But one cannot be sure that the salol is well mixed with the food in the stomach, and hence it may go out with the first portions or with the last. Again, in cases of stagnation of the stomach, bacteria there can split some of the salol, and since the mucus will aid in this even without the organisms Huber recommends to test not the time of the appearance but of the disappearance of the salicyluric acid, which should have ceased in from twenty-six to twenty-seven hours. The urine is therefore examined first twenty-seven hours after the meal, and if found, at intervals of three hours. Sahli recommends to determine both its appearance and disappearance. While this is a very gross test, it does give a certain amount of information. In cases with disturbed motility, especially in those with simple atony and pyloric stenosis, the test may not appear for several hours, and may continue for even forty hours.

Jodipin has also been recommended by Winternitz.[18] Pancreatic secretion and the bile are necessary to split iodine from this fatty compound.

To determine the amount of residue in the stomach various methods have been proposed, such as the introduction of 100 gms. of olive oil (Klemperer) and the removal of as much as possible in two hours, washing well with water and separating the oil in a separating funnel. This method is severely criticised since the oil does not mix uniformly with the contents.

Sörensen and Brandenburg[19] recommend to give on the empty stomach 300 to 500 cc. of 3 per cent. protogen. Of this there is removed as much as possible in from one-half to one hour. From 100 to 200 cc. of water are then introduced and again removed. The nitrogen in both fractions is determined by the Kjeldahl method, and from this the contents calculated.

As regards the composition of gastric juice in dilated stomachs, it will depend on the disease causing the dilatation. In general there are two groups of cases,—those with acid and those with anacid contents. The former occurs in cases of ulcer and of continuous secretion, the latter in cancer and chronic gastritis. In general the mucosa becomes less sensitive, owing perhaps to the constant presence of food and the gradual development of a chronic gastritis, hence the acidity reduces.

Of 45 of our benign casts, 7 were hyperacid, 15 showed normal acidity, 9 hypoacidity with, and 4 without, any free hydrochloric acid.

By "hyperacidity" is meant the secretion of abnormally acid gastric juice during digestion, that is, while there is normal stimulus for secretion. By "hypersecretion" is meant a secretion of gastric juice in amount out of proportion to the physiological stimulus, or when this is absent. Hyperacidity is qualitative, hypersecretion is quantitative, yet they usually coexist. Hyperacidity often exists without symptoms, is, in fact, often a constitutional anomaly; hypersecretion is always pathological and produces pathological results (Riegel).

Hyperacidity, Superacidity, Hyperchlorhydria, or Hyperaciditas Hydrochlorica.—This secretion of very acid juice during digestion, but not on the empty stomach, involves not alone the total acidity, but especially the increased free hydrochloric acid. There might, indeed,

[18] Zeitsch. f. physiol. Chemie, vol. xxiv.
[19] Arch. f. Verdauungskrankheiten, Bd. 2.

be a high total acidity due to a large amount of organic acids which would not come under this head.

This may be due to nervous causes, to defective nervous control, or to changes in the mucosa. After the Riegel meal the motility is found normal or even increased (hyperkinesis), and the stomach empty at the end of six or seven hours, and sometimes even in three to four hours, the food being discharged into the intestine before it is ready. The acidity per cent. is often 100, sometimes 150 to 160 or more following the meal, and over 70, and even 100 or more, with the test breakfast. The free acid after the meal is 60 to 80, after the breakfast from 50 to 60. Organic acids are absent. Others (Meunier) say the acidity alone is not important, but the specific gravity must be low, 1007 to 1019 instead of 1022 to 1040, as normally.

The digestion of meat is excellent, but not that of starch. Absorption is good. Some of these cases are certainly functional, while others are secretory neuroses. For this latter diagnosis all causes for gastric disease must be excluded, and the increased acidity should vary with the nervous symptoms and be very variable, hence the term "heterochylia" (Hemmeter), while in chronic gastric disease the acidity is quite constant. The hyperacidity may be present only during certain periods and following certain nervous stimuli, an important point in a diagnosis at best difficult.

Hypersecretion, Supersecretion, Continuous Secretion "Gastrosuccorrhœa."—In continuous secretion the secretion continues when the stomach contains no food. If the bread of the Ewald breakfast be given without the water, and much is removed, it means true hypersecretion due to a disproportion between stimulus and response—the free acidity is relatively high, which is a valuable point in ruling out motor insufficiency. Continuous secretion is determined by finding much acid gastric juice in the fasting stomach and without anything to indicate stasis. To exclude food the stomach must be well washed. This condition may be constant or intermittent, a part of a general neurosis, a secretory neurosis, or the result of organic nervous disease. Among the last may be mentioned the gastric crises of tabes dorsalis; among the first are cases of gastroxynsis (Rossbach).[20] It is seen in neurasthenia and hysteria, myelitis, general paralysis, and even the excessive use of tobacco. In the periodic or intermittent cases (Reichmann's disease), during the intervals the person's digestion may be perfectly normal. Then occur sudden pains, acid eructations, and the vomiting of a cloudy yellowish fluid, first with food, then pure, often several hundred cubic centimetres of fluid of normal or increased acidity, the latter usually only when food is present (total acidity 30 to 50, abundant free HCl).

[20] Deutsch. Arch. f. klin. Med., Bd. 35.

In the diagnosis of the chronic cases, many of which are considered as functional disturbances, all that is necessary is to find considerable gastric juice in the food-free and previously empty stomach, and in amounts over 100 cc. (Some consider 50 cc. a safe limit; and sometimes 1000 cc. are found.) Yet many now doubt any such pure form, considering that stasis always precedes and hence food is always present, also that all such cases are due to ulcer or its sequelæ, and that the motor disturbances resulting are more important than the secretory abnormality.[21] These chronic cases are of long duration with a gradual onset. There is discomfort, the feeling of weight, acid eructations, pain at the height of digestion, which increases until relieved by vomiting. Night vomiting is especially characteristic. The pain often occurs before the meals and is relieved by eating. From 500 cc. to 1000 cc. or more may be vomited in severe cases, of cloudy fluid, which on standing separates into three layers. The gastric juice is either normal or slightly hyperacid.

Riegel recommends in the diagnosis of these cases, first, the removal of the contents at the height of digestion after a full test meal; then on the following morning the fasting stomach is tested. On the third morning the tube is again introduced, the stomach having been washed the previous night. One must be sure by washing out the stomach that it was indeed clean, for food fragments easily remain. After the test meal one often gets over one litre of contents, with a total acidity per cent. of from 90 to 100, free HCl 50 or more (sp. gr. 1004 to 1006.5). A case reported by Thayer is a good illustration. It was of two years' duration; the total acidity after the Ewald breakfast was 113; the fasting stomach always contained even 420 cc. of acid fluid, acidity per cent. often 117; digestion was good.

In these cases dilatation of the stomach results, and sooner or later yeasts and sarcinæ may be found. As above said, some think that these cases are not true hypersecretion, but that the primary element is the atony of the stomach, which allows a certain amount of gastric juice to remain, which fluid serves as a stimulus to further secretion. Riegel considers that the atony alone is not sufficient, since in so many cases of motor insufficiency the stomach will remain empty after it is washed out, and not secrete more if some water is left in, hence some other factor is necessary to explain the secretion. Again, much of the motor insufficiency is the result of the hyperacidity and hypersecretion, which cause spasm of the pylorus, hence failure of the stomach to empty itself, and hence dilatation. In several cases of dilatation of the stomach relieved by operation the condition soon recurred, showing that the secretory abnormality was the basis of the trouble (Riegel). It must also be admitted that such stomachs

[21] Kaufmann, Am. Jour. Med. Sci., 1904, vol. cxxvii.

are abnormally sensitive to stimulus, while in simple dilatation with stasis the mucosa does not respond with normal irritability, but seems dulled by the constant presence of food.

Other such cases ordinarily called nervous are supposed to be reflex disturbances from the intestine and are relieved by treating this organ.[22]

Nervous Dyspepsia.—Hyperacidity, hypersecretion, anacidity, are conditions which may accompany a variety of disturbances, and as terms they refer only to the chemical composition of the gastric juice. These abnormalities of secretion may be due to organic changes of the mucosa, to functional disturbances following bad habits of eating, poor food, etc., or be a part of a general neurosis, "nervous dyspepsia." In this country the last is an exceedingly common manifestation of neurasthenia which stomach specialists abroad speak of as the "American disease." Yet it is exceedingly difficult to separate the element due to food, rapid eating, etc., from the neurotic element, and in the majority of cases perhaps both coexist. There is usually good reason for gastric distress, and a neurasthenic will often worry his subliminal gastric sensations into the sphere of consciousness.

Some of these neurasthenics, if one tests the gastric juice, show hyperacidity; more, slight subacidity; and many, normal conditions. It is interesting that the subjective features bear so little relation to the condition of the gastric juice, a patient with hyperacidity describing sometimes quite similar sensations as an anacid case, unless vomiting also occur which the latter is, as a rule, spared; and one with apparently normal gastric juice sometimes complains as much as either of the others.

In this clinic during the past four years we have had 300 such cases. We have made no effort to separate "functional" cases from the purely neurotic. Eighty-two were cases of hyperacidity (this includes the cases of supersecretion and continuous secretion; all figures quoted are those of the Ewald breakfast). Of 20 others the clinical features were hyperacidity, although the total acidity was not over 70. In 36 cases the total acidity was 70 to 80, the free 33 to 69 (the majority from 45 to 55); in 21, from 80 to 90, free acid 32 to 68 (the majority 55 to 65); in 15, from 90 to 100, free acid 53 to 85; in 10, from 100 to 110, free acid 60 to 89. As regards amount of fluid obtained one hour after the test breakfast, over 100 cc. were obtained in the first group (total acidity 70 to 80) in 30 per cent. of the cases; in the second group, in 35 per cent.; in the third, in 20 per cent.; while in the group with total acidity over 100, in 29 per cent.

Subacidity (total acidity less than 40) was present in 170 cases, in 61 of whom there was no free hydrochloric acid. In these 61 cases the total acidity was seldom over 20, in 18 was 10 or less, and in 4 the fluid was practically neutral to litmus. It is of interest that in these subacid cases in but 4 per cent. was more than 100 cc. obtained, while in 8 per cent. nothing could be siphoned off at the end of one hour. In 148 cases the gastric juice was found practically normal. This may illustrate the lack of parallelism so often seen between the sensations and the chemical findings, but one must also consider the possibility that

[22] Faber, Arch. f. Verdankhtn, Bd. 7.

the test breakfasts were not given at the best time to observe the abnormality in secretion.

Acute Gastritis.—By this is meant an acute irritation or inflammation of the superficial layers of the mucosa, resulting in increased mucus secretion, or desquamation of the epithelium, and disturbance of secretion. It may be primarily due to the direct irritation of foods, poisons, or intoxications, heat, cold, etc., or, secondary to various chronic diseases. The vomitus of these cases is acid in reaction, of a bad odor, often fermented, the food undigested as a rule and with much mucus. The total acidity is diminished, free hydrochloric acid absent as a rule, and often organic acid present. Rarely is the reaction neutral. If there has been much retching, the vomitus is bile-stained or even pure bile. A test meal will show mucus, undigested food, and little or no free HCl.

We have records of but five good cases, all with subacid or neutral fluid.

Gastritis phlegmonosa or interstitial purulent gastritis is a very rare condition. It is an inflammation of the entire gastric wall even to the serosa. When localized it gives rise to gastric abscess. Vomiting is present, as a rule. In the 60 diffuse cases reported, however, pus has not been present (Riegel), but has been in a very few cases of abscess.

In the gastritis acuta purulentia (Leube) the inflammation is limited to the mucosa.

Chronic Gastritis.—Chronic gastritis is not nearly as common as is its diagnosis. It exists in all grades to atrophy of the mucosa. Functional disturbance must first be excluded, and only those cases included in which there are definite signs of gastritis with increased mucus formation. One of the commonest symptoms is vomiting, especially on an empty stomach in the morning or at the height of digestion, of undigested or poorly digested food mixed with mucus. If on the fasting stomach, it consists of bile-stained mucus, well seen in the morning vomiting of alcoholics.

The test meal must be tried. The amount removed is about normal. The food has the appearance as if just swallowed, and much mucus is present which is intimately mixed with the food. This renders its removal through the tube difficult and its filtration tedious. The presence of this mucus is indispensable for the diagnosis, since large amounts from the stomach indicate catarrh. The macroscopic appearance is the best standard for amount. To judge the amount of mucus, however, the stomach must be thoroughly washed, since the most appears in the later washings. Hence it is that the vomitus is so often

deceptive in this particular. The needle douche tubes are often valuable.

The secretion is usually diminished, and in late cases with atrophy of the mucosa there may be no secretion at all. Free hydrochloric acid is diminished or absent, but the total acidity varies and for short times the free acid may return, hence the necessity of repeated examinations. There are cases of acid gastritis, but they are rarely diagnosed, though this may be due to the fact that they are an early stage before the patient consults a physician.

Of our 27 cases, one was slightly hyperacid (72, total acidity); in 10 the acidity was within normal limits, and in 15 below 40, nine of whom had no free acid. Four of these could be termed atrophic catarrh, and one additional case, from which stomach could be obtained by the tube but 1 cc. or more of bile-stained mucus, at autopsy was found to be a case of cirrhosis of the stomach.

For the diagnosis of a gastritis acida it is necessary to find in the fasting stomach mucus with many cell nuclei and a hyperacid juice.

As a rule, there is diminished secretion, and as the case progresses the secretion becomes less and less; pepsin is also reduced. Organic acids are present when there is considerable atony, and then only in small amount. Proteid digestion suffers, yet there is usually enough gastric juice to digest some. Starch digestion is not disturbed; the rennet is diminished as well as the pepsin, and is considered by Bouveret as a good criterion for the intensity of the case and for prognosis. The amount of fermentation will depend upon the motility. Einhorn considers that in chronic gastritis one commonly finds fragments of the mucosa in the wash-water. Motility is sometimes normal, sometimes increased, or sometimes diminished. If normal or increased, the intestine will act vicariously and hence very few symptoms be present. If decreased, these are more severe, and yet, from the presence of undigested food at a time that the stomach should be empty, one cannot conclude that motility is disturbed, since normally only food which is digested to a certain point is allowed to pass into the intestine. Again, in such cases there is often a slight obstruction at the pylorus due to the inflammatory swelling of the mucosa.

Mucus.—Mucus in the stomach washing is present in delicate transparent flakes, never in balls, is mixed with the food, and sinks in water. These flakes may not be present in the vomitus or in the siphoned fluid, but may be obtained in abundance if the stomach be well washed out, especially if a needle douche tube be used; hence from the meal or vomitus alone a wrong opinion concerning the amount of mucus is sometimes obtained. The mucus from the respiratory passages is in balls, glairy, usually contains air, is therefore frothy and swims in the water, often contains epithelial cells and pigment which will disclose its origin. It is not mixed with the food. The gastric

mucus contains the nuclei of leucocytes. When digestion is poor these cells may remain well preserved. The presence of mucus in the washings may not indicate an increased secretion, since that normally secreted may not have been digested because of the lack of hydrochloric acid. Mucus is normally increased on a starch diet, *e.g.*, yet so little is present that one must hunt for a few flakes even in the centrifugalized washings. A great increase of gastric mucus indicates a gastric catarrh. Mucus is normally digested by the gastric juice one-half as fast as is albumin. Again, when there is a little acid present the mucus may swell and hence appear large in volume. It is remarkably increased in carcinoma of the stomach and in chronic gastritis with atrophy of the mucosa. The reason for its increase is in considerable doubt, and is attributed to an abnormal stimulation of the mucous cells of the glands, which after all secretion of pepsin and acid has ceased will continue to form even more mucus than normal. In some cases with hyperacidity there is an increased mucus production, but this is not the rule, for in general mucus and hydrochloric acid vary inversely. To get some idea of the amount acetic acid should not be used, since the partially digested mucus is not precipitated in this way.

Atrophy of the Mucosa. Achylia Gastrica.—Achylia gastrica may be due to a functional disturbance of an apparently normal mucosa or to real atrophy of the mucosa; and the latter, the end stage of a chronic gastritis or the result of cancer and other diseases which lead to degenerative changes in the mucosa. When due to atrophy it is a gradual process, the secretion diminishing until finally there is almost no gastric juice. The diminished secretion may be due to a very small cancer, and much of the mucosa seem intact; it seems due to some toxic substance from the tumor. Achylia gastrica can exist when there is cancer in other organs (breast, intestine, œsophagus, uterus, *et al.*), and before any disturbance of the general health. In conditions with general malnutrition also it may be present. Those cases of particular interest resemble pernicious anæmia, and yet the local trouble may not be suspected provided the motility of the stomach be good, and sometimes it is increased, yet if slight atony exist also the symptoms will be evident enough. If the motility is good the intestine will act vicariously.

Some cases are of nervous nature. Einhorn reports such a case of five years' duration of achylia and then a return to normal. The discovery may be purely accidental. On the other hand, the nervous symptoms may disappear, leaving the achylia still in evidence.

Atrophic stomachs are very susceptible to injury, and it is not uncommon in the washings to get pieces of mucosa which seem to show a granular gastritis. Such cases vomit often, not always, and,

as a rule, soon after eating, the vomitus consisting of undigested food, and is almost never bloody. For diagnosis of achylia the test meal is necessary, and this perhaps is the condition in which the test meal gives the most positive results. It should be repeated several times. It is impossible to remove much. The food is little changed, the total acidity is very slight, from 1 to 4. There is no free HCl. Lactic acid is rare unless there be severe ectasis. To diagnose the anatomical condition is more difficult. The ferments may fail, an important point in diagnosis. It is easy in washing out the stomach to get fragments of mucosa and traces of blood showing the vulnerability of the mucosa. Mucus is, as a rule, absent, yet early there may be much, and later the mucosa may consist chiefly of mucous cells, the glandular cells having disappeared. To obtain nothing through the tube does not necessarily mean an empty clean stomach, since by washing the dry contents may be removed. Elsner,[23] *et al.*, consider the vicarious action of the intestine to be overrated; undigested food should be retained, and if the stomach is washed out this will be found.

Elsner's method of measuring motility is to wash the stomach out well one hour after the test meal. This fluid is allowed to stand in a graduated cylinder for twenty-four hours, then the amount of residue read after several decantings. If there is over 210 cc. of residue there is motor insufficiency in addition to the achylia.

Ulcer of the Stomach.—The clinical types of this disease are:

(1) The latent form which may pass unsuspected or until hemorrhage or perforation occurs.

(2) The hemorrhagic form, which may be acute and sometimes fatal, or chronic, causing considerable anæmia and cachexia resulting from the frequent small hemorrhages, the stools always containing a certain amount of blood.

(3) The acute perforative.

(4) The chronic dyspepsia, in which case the dyspeptic symptoms are the most evident, and the characteristic symptoms of ulcer vary, the most important chemical signs being the hyperacidity and the absence of mucus.

(5) Neurotic, or gastralgic type.

(6) The vomitive form, with vomiting as the worst symptom.

(7) The cachectic form, which presents the picture of a cancer.

The cardinal symptoms of this disease are: (1) Increasing dyspepsia, usually of long duration. (2) Pain, paroxysmal and local, from half an hour to two hours after the meal when peristalsis is most actively rubbing the food across the ulcer. (3) Vomiting of well-digested food and acid vomitus, usually one to three hours after the meal at the height of the paroxysm, but often in the morning also

[23] Deutsch. med. Wochenschr., 1904, No. 42.

since the juice is then hyperacid, and followed at once by a diminution of the pain. (4) Blood is only at times present, and in from 30 to 50 per cent. of the cases. As a rule, it is dark in color, due to the hæmatin formed by the hydrochloric acid, although if the stomach be empty it may be arterial. There is often blood in the stools intimately mixed with the food, but not as constantly as in cancer. In case the blood rests a long time in the stomach, the vomitus may be of coffee-ground appearance, in which case iron, wine, and coffee of the medicines and food must be excluded. In many cases the blood of the stools is unsuspected. Other sources of hemorrhage must be excluded,—tuberculosis, cancer, chronic passive congestion, cirrhosis of the liver, rupture of an œsophageal varix. (5) Hyperacidity, a classical symptom which, Ewald says, is present in but about half the cases. The digestion is good and motility usually rapid. In an average of 75 cases tested with the Riegel meal he found the total acidity to average 105, the average free HCl 50, with a maximum of 89. But the acid may be diminished, due to later disease of the mucosa, catarrh, etc. The two groups must be distinguished of the fresh and the old ulcers, and in the latter cases much lower acidity may be expected.

The result in such cases may be stricture, or severe anæmia due to the insufficient nutrition, vomiting, or hemorrhage. In case the blood is lost chiefly by the stools the ulcer may be unsuspected and the case be diagnosed as pernicious anæmia. Cancers may develop on the bed of the ulcer,—in fact, some think the majority of cancers begin thus,—and at the time of death the acid still be considerable. In most cases it gradually diminishes until the picture is typical. One examination of the gastric juice is never enough. Repeated examinations must be made in order to get a general idea of the acidity. In the case of developing cancer it is the variable yet diminishing acidity which is important, and yet cases of ulcer may have a normal or diminished amount of hydrochloric acid.

The 82 cases of this clinic have been reported by Howard.[24] Vomiting was present in 85.3 per cent., especially in the cases with ulcer at the pylorus. Vomiting of blood occurred in 75.6 per cent.; in one-third only was the blood bright red. After the test breakfast more than 50 cc. was obtained from 54 per cent., 27.5 per cent. showed hyperacidity, 42.5 per cent. subacidity (for these figures the acidity per cent. of 60 was considered the upper limit of normal). In 18 per cent. there was no free hydrochloric acid, in 14 per cent. lactic acid.

Duodenal ulcer is often impossible to diagnose. Yet the position of the pain, its late occurrence after the meal, and the fact that all the blood will appear in the stools is suggestive. Hyperacidity may be present. These ulcers are more often latent than are the gastric.

Hemorrhagic Erosions.—It is doubtful whether there is for this

[24] Am. Jour. Med. Sci., December, 1904.

a characteristic complex which as yet can be recognized. The most valuable point is that in washing the empty stomach there have been found usually fragments of the mucosa without marked pathological changes. Vomiting is rare. The acidity is normal or diminished, rarely increased.

Cancer of the Stomach.—Clinically these cases may be separated into the latent, those with cachexia but no gastric symptoms, and those with localizing symptoms. The important diagnostic points of this disease are its rather sudden onset with dyspeptic symptoms in a person beyond middle life, unless it be a case that develops on the base of an ulcer the symptoms of which have long preceded it; loss of weight and strength; anæmia; pain; vomiting; and on chemical analysis lack of free hydrochloric acid, the presence of lactic acid and of the Oppler-Boas bacillus.

Of these local symptoms and signs, however, any one may be absent. Vomiting is common (in 85.3 per cent. of our first 150 cases), yet it depends upon the position of the cancer; if at the pylorus causing stenosis, the vomiting will be late after a meal; if at the cardiac orifice, there will be regurgitation at once after eating. There is least vomiting when the cancer is on the stomach wall. If there is no stenosis at either orifice, there will often be none, yet in 6 of 30 cases with the cancer at an orifice there was none. In those cases with dilated stomach due to stenosis the amount of vomiting is often from a half to one litre or more of food, in which may be recognized that eaten days before, in one of our cases four weeks (Osler and McCrae). The albuminous part of the food is poorly digested, hence the meat in lumps, with mucus often, sometimes decomposed and often with digested blood. Some hemorrhage is almost constant. It is parenchymatous as a rule, and yet it may be rapid and fatal. It is often an early feature, and leads to the diagnosis of ulcer. Often the patient will not know of the hemorrhage unless his stomach be washed out or his stools be carefully examined, since it is gradual and the blood by digestion takes on the so-called "coffee-grounds" appearance and is mixed with food. Of the 150 cases reported by Osler and McCrae, vomiting of blood occurred but in 21.8 per cent., but careful examination of the stools showed it present in almost 100 per cent.

The gastric features of cancer may be grouped as follows: First, those due to the pyloric stenosis, with subsequent dilatation of the stomach and stasis of the contents, hence fermentation, decomposition, etc. In some other cases the motility is excellent or even increased, as in 11 of 76 cases, in which it was hard to get any fluid at the end of an hour. Second, those due to the chronic degenerative changes of the mucosa which begin early and develop late; the gradual diminution in the amount of secretion, *e.g.* And lastly, those due

to the cancer itself, the early absence of free hydrochloric acid, perhaps the presence of lactic acid, perhaps the presence of the long bacilli in chains.

To consider first the symptoms due to the cancer *per se;* absence of free hydrochloric acid is an early and important sign of cancer, present in over 80 per cent. of cases at first examination, yet alone not of great importance, not even when other signs are present; in cases of pernicious anæmia, for instance, there are many features of cancer, and in gall-stones with the pylorus in a mass of adhesions, and hence palpable tumor, the free HCl may fail. In 163 cases of this clinic the free acid was absent in 146, or 89 per cent.

Again there may be a group of cases without previous symptoms of ulcer which begin with hyperacidity (Ziegler). The total acidity may not be in the least diminished and total chlorides be high. The acidity varies considerably from day to day, and, in fact, even with the presence of free acid a variable acidity, with sometimes free hydrochloric acid and another day none, may lead to the diagnosis of carcinoma.

This was well seen in a recent case early in the disease, and was a point leading to operation. In fourteen days, of the three Ewald breakfasts given the acidities were total 121, 100, 37, and free HCl 11, 16, and 10 respectively.

The lack of free acid is at first certainly due to the binding of this acid by some body which itself does not react alkaline to litmus, yet which prevents the hydrochloric acid from giving the Günzberg or other tests for it in its free state. The idea originally suggested by v. de Velden, that there was a secretion of the cancer which bound the acid, is the idea now again advanced. Later, as a result of the changes in the mucosa, brought about perhaps by this secretion of the cancer, the total amount of acid will gradually diminish to a very small amount; but it is to be emphasized that the conditions causing its absence vary early and late. Early with normal total acidity absence of the free hydrochloric acid is one of the most important signs; later the amount secreted is diminished. In these early cases it is very interesting that the disappearance of free acid may be sudden, and that following the excision of the cancer the free hydrochloric acid has returned a day or two later; again, in cases of carcinoma of the duodenum or œsophagus the disappearance of free hydrochloric acid can be best explained as due to fluid from the tumor which flows into the stomach; the cancer may be small and very local, but its effect considerable. What these bodies are which bind the free hydrochloric acid has been a subject of considerable investigation. Certainly hydrochloric acid, if introduced into the stomach of a carcinoma case, is soon neutralized (Stahelin). They

might be the products of albuminous digestion,—albumoses and peptones. The hexone bases would have this same power and have been demonstrated in the products of peptic digestion of even half an hour's duration; Reissner ascribed the lack of free acid to the secretion of mineral alkalies from the tumor, but this is unsatisfactory, since the resulting chlorides would react neutral, and hence reduce the total acidity, while in typical cases of early carcinoma of the stomach the contents show a total acidity which is not diminished and may be higher than normal, yet with no free acid. It is true that evidence is given that the dog can secrete sodium carbonate, by this mechanism keeping the acid at a physiological level. From work which we have done in this connection we believe that the bodies in question are the hexone bases, the result of the digestion of the proteid by a ferment furnished by the tumor itself.[25] Later the acidity is much reduced, as shown by the increasing acid deficit and the decreasing total chlorides, and in some cases the fluid may even be alkaline to litmus, as its ash usually is. Or, as a result of lavage and general treatment, the acidity may be increased so that the case in which free acid was absent may later show an abundance. The failure of free hydrochloric acid is usually a very early symptom. In cases of cancer developing on the base of an ulcer this acidity may be above normal at first. The real disappearance of hydrochloric acid is gradual, but early occur daily variations of the free of much diagnostic import.

Of 64 of our cases without free hydrochloric acid, the juice after the Ewald breakfast was almost or quite neutral in 8, below 10 (acidity per cent.) in 20, between 10 and 20 in 15, between 20 and 50 in 14, and between 60 and 103 in 7. The high acidities seemed to depend on the lactic (and butyric) acid present.

Later the pepsin is diminished and the rennet as well. This is due to the chronic gastritis resulting in a diminished secretion of gastric juice, and is not specific for the cancer.

Lactic acid is often an early and very valuable sign in cancer. It occurs in about 90 per cent. of the cases sooner or later, when there is no free hydrochloric acid although the bound acid may be abundant. It is true that cases of cancer without lactic acid occur, *e.g.*, those on the base of an ulcer; also more rarely lactic acid without cancer, as in case of an atonic and anacid stomach, atrophic catarrh with stenosis of the pylorus and hence long stagnation of the gastric contents; but in the benign cases it is so often absent, even though the stenosis be extreme and the fluid anacid, that its early appearance in cancer, even before the stenosis is considerable and the total hydrochloric acid much diminished, means that these two factors cannot alone explain its appearance, and emphasizes the value given it by Boas in the early

[25] Arch. f. klin. Med., vol. lxxii. p. 415.

diagnosis of malignant disease, although the specificity he claimed is not granted.

In 609 of our cases without gastric cancer, lactic acid was present in 30. All were cases of subacidity with no free hydrochloric acid. These cases were: atrophy of mucosa, 1; chronic gastritis, dilated stomach, 4; ulcer, 6; nervous dyspepsia, anacidity, 3; pernicious anæmia, 2; gall-stones, 1; cirrhosis of liver with jaundice, 1; cancer of gall-bladder, 1; pulmonary and peritoneal tuberculosis, 3; and an interesting group of inflammations of the large intestine (ulcerative colitis, etc.), 5; cancer of ovary, 1; peripheral neuritis and fibrinous pericarditis, 1 each.

Riegel considers the chief cause to be the motor insufficiency with subacidity; that for its appearance there must be, first a diminished secretion involving ferments as well as acid, then stagnation of the contents, and perhaps insufficient absorption. Yet he admits that there may be considerable lactic acid in a case without any great atony, and that as a result of twenty years' experience excepting in very rare cases large amounts always meant cancer. He explains one case without stasis by the fissures at the base of the cancer, which allowed the retention of acid-producing organisms. Hammerschlag considers that it appears only when the ferments are diminished, hence when proteid digestion is poor, and is a sign of the diminution in the secretion of gastric juice with stagnation and deficient absorption. This occurs particularly in carcinoma of the pylorus.

While the lactic acid may often be determined in a test breakfast, this is hardly a fair test since its formation cannot be as rapid as that of the secreted acids. It is best therefore to test the contents of the fasting stomach in the morning after it has been well washed out the evening before and a test meal given (see page 336).

The cause of the lactic acid may be the organisms in the stomach, since several of these have been proven to be acid-producing, among them the Boas bacillus; or it may be a normal product of digestion evident in these cases because of diminished absorption (an improbable explanation to cover many cases); or it may be the product of a specific ferment furnished by the tumor. This latter cannot be excluded, and in the autolytic digestion of proteid by ferments from these tumors lactic acid has been shown to arise.

In our cancer cases the fluid removed one hour after an Ewald breakfast was examined for lactic acid, hence the per cent. which it presents will be minimal. It was present in 63 per cent. of 137 cases. The figure given by Schiff was 73.5 per cent. of a group collected from various writers.

The gross appearance of the contents is of great importance, since the meat is poorly digested and the carbohydrates well. Disturbance of motility is due to mechanical obstruction at the pylorus. On the other hand motility may be excellent and yet digestion very poor,

which is true in early cancer not situated at the pylorus. Strauss claims in such cases we have an abnormal fermentation due to bacteria which have remained in the clefts of the tumors.

Tumor fragments are seldom found. Fragments in blood-clots washed from the stomach should be examined. Sahli emphasizes the possibility of diagnosis from washing out the stomach well at night and the fasting stomach again the next morning; in the latter wash-water the fragments of the tumor may be found. Considerable blood in various stages of digestion is common. In achylia gastrica fragments of mucosa may easily be washed loose and resemble cancer. In this clinic fragments were found in several cases, but the number depended on the care with which they are searched for, for in over 70 cases they were noted but twice, and in a few months after the attention of the clerks was called to the need of searching for them several cases were found.

Sarcinæ and yeasts are rare. In but five cases of our clinic were sarcinæ found. The bacteria of the stomach, which are probably a large

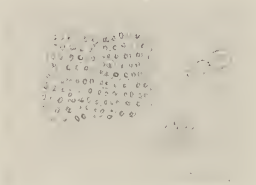


FIG. 64.—Sarcina ventriculi and yeast cells. × 900.

group, have been divided into the "short" and the "long." The former occur in catarrh and ectasis due to benign conditions. The presence of many sarcinæ is evidence against cancer. That which has attracted the most attention is the so-called Oppler-Boas bacillus, which occurs in about 80 per cent. of cases (Rutinmeyer), and in almost no other condition than here. Cultural characteristics of this organism have not as yet been well worked out, and the cultures are so seldom made that as the Oppler-Boas bacillus we usually have in mind a group of organisms with a few points in common, especially the morphological characteristics; that is, a long, coarse, thread-like bacillus, often in long chains which extend across the field of the microscope, in some cases present in enormous numbers even filling the whole field. No spores are seen. The single bacilli are from 3 to 10 microns long (6 to 8 microns as a rule) and 1 micron broad, with rounded ends, often slightly bottle-shaped; some are bent. They stain by Gram's, and are best seen in stained specimens. Of course, few or many could be present, but we never speak of them as Oppler-Boas bacilli unless

they are abundant, coarse, and in chains. Some passing under this name are surely the Gas bacillus, but the Boas bacillus is not anaërobic, and has been found to grow best on media containing blood or its derivatives, and hence perhaps its presence so often and so exclusively in carcinoma, in which condition above all is present the coffee-ground vomitus rich in albumin detritus, the ulcerations and clefts of the mucosa, the failure of ferment formation, of acid secretion, and the stagnation, which factors Schmidt considers essential. These bacilli do not grow well on ordinary media, but will luxuriantly if blood be added. They coagulate milk. Kauffmann found the bacillus in 19 of 20 cancer cases, proved that it was a lactic acid builder, and found that it occurred in numbers proportional to the amount of lactic acid. Most other lactic acid bacilli are smaller. Other bacilli of similar appearance have been cultivated,[26] yet its diagnosis is chiefly morphological. Apropos of the number present, a recent case without extreme stasis may be mentioned, for the gross sediment of the stomach washings was almost entirely composed of masses of these bacilli. It is not fair to search for them in a recently washed stomach. If there certainly is stenosis and these are absent, the evidence is against cancer. Kaufmann [27] claims that they cannot grow in the presence of free hydrochloric acid of 0.02 per cent., but can well when the fluid is acid with phosphates and lactic acid; but others (Rosenheim) say they flourish in the stomach in spite of free hydrochloric acid.

Our series is hardly the right one to furnish statistics concerning the presence of these organisms, for only the fluid removed after an Ewald breakfast on a cleaned stomach was examined, yet these organisms were found in only 38 per cent. of 55 cases in which their presence or absence was noted. In four of these cases with the bacilli lactic acid seems not to have been present.

Heichelheim [28] thinks in diagnosis that clots of blood are very important. To find clots containing many of these bacilli in a fluid without free hydrochloric acid speaks very strongly for cancer; clots with few bacilli strongly suggest it; clots alone and repeatedly present speak in favor of it.

Pus is sometimes present; in fact, the largest amount of pus that we have seen in any gastric case was one of carcinoma. The resorption is much disturbed and the KI test almost always delayed.

Among other tests proposed for the early diagnosis of cancer of the stomach is the tryptophan test of Erdmann and Winternitz [29] which is not constant enough (Sigel in 2 of 15 cases; Glässner in 1 of

[26] See Schmidt, Wien. klin. Wochenschr., January 10, 1901.
[27] Centralbl. f. inn. Med., 1904, No. 4.
[28] Zeitschr. f. klin. Med., 1904, vol. liii. p. 447.
[29] Münch. med. Wochenschr., 1904, p. 299.

2) to be of great value and does occur in other conditions (ulcer, *e.g.*), yet it makes the diagnosis very probable (Orlowsky).

The presence of over 0.5 p. m. of albumin (Esbach), which Salomon considered the surest sign, and of urea in the washings of a fasting stomach without retention, are of some but not absolute value. The stomach is carefully washed out; then in a few hours the tube is again introduced and all possible removed, washing several times with 400 cc. of physiological salt solution. The albumin and nitrogen are then determined. In all other conditions N = 0 to 16 mg. per 100 cc., but in cancer 10 to 70 mg. per 100 cc.: cancer is probable when N = more than 20 mg. and there is a definite albumin precipitate by Esbach's fluid. Common ulcer can be differentiated early, although it is the ulceration of the cancer nodule which furnishes this inflammatory exudate.[30]

Gluzinski's test for the relative insufficiency of HCl secretion by testing free hydrochloric acid in the morning on the fasting stomach, forty-five minutes after the test breakfast, and four hours after a full meal, is valuable also to indicate a cancer on the bed of an old ulcer, it being positive in 12 of 13 cases.

Infusoria, and especially flagellates, are sometimes present in the anacid carcinomatous stomach at a very early stage. Cohnheim [31] reported six cases with the Trichomonas and Megastoma entericum, and thinks this a valuable sign, even the first, for the early diagnosis of an ulcerating cancer of the cardia or lesser curvature; not in pyloric cancer, since the lactic acid would kill them. They are often present in our food and are a temporary inhabitant of the stomach until acid is excreted.

Zabel [32] reported four early cases with similar organisms present in abundance. Rosenfeld [33] found them in six cases, one of which he thinks is the first non-carcinomatous case in which they have been found. He expected this would be true of another case, but a cancer was later in evidence. They are found in the small amount of neutral or alkaline fluid of these fasting stomachs, together with leptothrix threads, long bacilli, and spirilla. It is interesting that they cannot be found in other cases of achylia, for we must often swallow them.

Blood in the gastric contents and stools is a very important, common (68 of 70 cases),[34] and early feature, especially in the absence of hydrochloric acid and when motility is good.

Strauss emphasized the disproportion between the relatively active fermentation and small amount of sediment in case the cancer is not at the pylorus; Reissner, the early increase of chlorides to almost or

[30] Berent and Gutmann, Deutsch. med. Wochenschr., 1904, No. 28.
[31] Deutsch. med. Wochenschr., 1903.
[32] Wien. klin. Wochenschr., 1904.
[33] Deut. med. Wochenschr., 1904.
[34] Boas and Kochmann, Arch. f. Verdauungsk, 1902.

quite double, and the alkaline reaction of the ashed gastric contents. For Glässner's idea concerning ferments see page 326.

The early diagnosis of cancer of the stomach is unfortunately a late one if one means by "early" a diagnosis made in time that operation can save the case. No one feature will help for even a fairly early diagnosis. The chemical features may be very suggestive, in some cases normal, in some even the reverse of those suggesting cancer. The surgeons insist that the diagnosis should be made before any tumor is palpable, and this should be the aim of the clinical chemist. At present we admit that age and clinical history are of far more importance than chemical examination, and would never delay operation until the clinical chemist found positive any test proposed till yet, fearing that were it positive it would then be too late to operate.

CHAPTER IV

THE INTESTINAL CONTENTS AND FÆCES

To determine the **motility of the intestine** is often important, particularly in metabolism experiments to separate the stools belonging to the various periods; also in "latent constipation," one of the "new" diseases, in which the food is much too long in its passage through the intestine, but the condition overlooked since the stools are normal in number and size. It would indeed be fortunate did this condition remove the stigma of neurasthenia from some of our suffering patients. The normal motility after a mixed meal is from six to twenty hours; after milk, thirty-six to forty-eight hours. Charcoal or lycopodium powder is generally used, one drachm in water after a meal and the stools watched until the black charcoal is seen grossly, or the characteristic lycopodium spores microscopically. Or carmine, 0.5 gm., may be given and the red color watched for. Allowance should be made for the gastric motility in case the actual time in the intestine is desired. Sometimes the charcoal is so mixed in a considerable mass of fæces that it passes unnoticed, hence lycopodium or carmine is somewhat safer.

Pancreatic Fluid.—This when present in the duodenum may be obtained, according to Boas, by massaging the contents of the duodenum into the stomach previously washed with 1 per cent. soda solution. The patient lies on his back, and the abdomen is massaged from right to left from the costal margin to the parasternal line. The stomach-tube is then quickly introduced and whatever may have been forced back removed. Sometimes about 50 cc. are obtained. To prevent the destruction of the ferments by the hydrochloric acid, soda should be added at once. The presence of trypsin is assumed if fibrin or egg albumin is digested in alkaline medium.

Trypsin. ARTHUS AND HUBER'S METHOD.—Fresh fibrin is washed in water and then heated at 40° C. for twenty-four hours with 2 per cent. NaF. The solution of fibrin is then filtered. The intestinal fluid plus an equal amount of 2 per cent. NaF is mixed with two to three volumes of the above-mentioned fibrin solution and kept in the thermostat at 40° C. for some time. If trypsin is present the typical crystals of tyrosin will be easily found. Contamination with bacteria is not to be feared, for the fluid will remain sterile indefinitely.

The FAT SPLITTING FERMENT may be demonstrated as follows: Neutral olive oil is obtained by shaking out olive oil with ether and a little NaOH. The ether extract is shaken out repeatedly with water

and the ether then evaporated. An emulsion is then made of oil 10, gum 5, and water 35 parts. In several test-tubes (12 mm. diameter) are then mixed, litmus solution (neutral and dilute till of a violet color against a white background) 10 cc., the emulsion 5 drops, and then the fluid to be tested 2, 4, 8, and 16 drops in each respectively. The tubes are then put into a water bath (37° C.) at once, and examined in a few minutes for the red color.

To test the DIASTASE the fluid is added to a thin starch solution. Soon dilute iodine solution added to a drop of this will give no longer a blue color.

Test-Meals.—All recognize now the necessity of using standard test-meals for the examination of intestinal conditions. Strauss recommends one of chopped beef 100 gms.; others one of milk. The bowel should first be well purged and charcoal, carmine, etc., then given to mark the period.

The Digestive Power of the Pancreatic Juice.—A method which has promised much is that of Sahli, who gives with an Ewald test-meal 0.15 gm. of iodoform in a glutoid capsule (gelatine hardened in formalin). This is supposed to be digested only by the pancreatic juice; iodine will appear in the sputum in from a quarter to one and a half hours after the solution of the capsule. The sputum is examined hourly with nitric acid and chloroform. It is important to know the condition of the stomach first, and the result depends not only on the digestion of the capsule, but on the absorption of the iodine. To determine the motility of the stomach Sahli gives on the following day with a glass of water on an empty stomach the same amount of iodoform in capsule which has not been hardened, assuming that this passes at once to the intestine.

Sahli's results are interesting: In cases without hydrochloric acid or pepsin, if the motility be good the test indicates no abnormality, showing the vicarious action of the intestine; in cases of diarrhœa due to increased peristalsis alone the iodine appears in a normal or diminished time; in diarrhœa with disturbed chemistry or absorption it may be delayed or even the capsule found in the stools. In some cases of jaundice with obstruction at the ampulla there is delay, yet occlusion of the pancreatic duct cannot be excluded on the ground that the test was positive in normal time, since the pancreas often has two ducts. If in pancreatic disease the test is negative, it is in favor of cancer, although not much weight can rest on this point, and Galli[1] condemns it, since in a case of carcinoma of the pancreas without any pancreatic juice at all the capsule was promptly dissolved.

Unfortunately, Sahli does not give very explicit directions concerning the preparation of these capsules, and it would seem that they

[1] Deutsch. med. Wochenschr., 1903, No. 19, p. 716.

could be obtained from but one source. This is unfortunate, yet it is for those interested in the success of the test a wise provision, since so much depends on uniformity in the preparation of the capsules.[2] For the pancreon test, see page 391.

In cases of *jejunal fistula* it is often of importance to know how near the pylorus is the opening. A convenient method is to tie a silk thread to an oyster. In these cases of practical starvation with the fistula high up, the motility of the stomach is excessive, as if that organ were trying to aid the body by sending the food at once to the intestine. In an interesting case in this hospital[3] the oyster appeared at the fistula's orifice, which, by measuring the length of the string, was found to be but one foot below the pylorus. In this same case, after drinking a glass of milk the milk began to escape from the fistula in one minute, and the total amount was recovered in four minutes.

The examination of the stools is much neglected. It is disagreeable but so valuable that it should never be overlooked. As the sputum examination is commonly limited to staining for the tubercle bacillus, so that of the fæces is now a matter of searching for parasites when they are suspected, with the result that much that is valuable passes undiscovered, and, when examined, the ordinary constituents, since not familiar, are misinterpreted.

For this examination are necessary a few tall glass jars in which the stools mixed with water are allowed to sediment, some strainers (colanders) of various sized mesh in which the stool is ground by a pestle, and plates half black half white, the same as used for sputum.

The **constituents of normal stools** are the undigested portion of food, bacteria, intestinal secretion, formed and unformed elements from the mucosa, salts, and products of digestion. The amount varies widely with the diet, but a general average is 120 to 250 gms. per day.

The relative amount which bacteria form is enormous. Strassburger[4] estimated them at about one-third the weight of dried stools, that is, eight grammes per day, and containing about one-half the nitrogen of the stools. He found more in some dyspepsias,—14 to 20 gms.; remarkably less in chronic constipation,—5.5 to 2.6 gms. Strassburger's method was as follows: 2 cc. of the stool is well mixed with water and centrifugalized; the organisms will remain suspended, the elements of the food sediment. The fluid is then poured off, considerable alcohol added to lower the specific gravity, and again centrifugalized. This time the bacteria will sediment. This sediment is then dried and weighed. Another 2 cc. are evaporated (fresh alcohol

[2] Sahli. Deutsch. Arch. f. klin. Med., 1898, Bd. 61.
[3] Cushing. Johns Hopkins Hosp. Bulletin, July, 1899.
[4] Zeitschr. f. klin. Med., 1903, Bd. 48, p. 413.

being repeatedly added), dried and weighed. Klein,[5] who uses a counting method but who does not even guess the relative volume of the bacteria in the stool, takes exception to Strassburger's method and results.

The small amount of fæces during starvation periods consists of bacteria, the intestinal epithelium, mucus, and the intestinal secretions.

Reaction.—The normal reaction is neutral, faintly acid, or faintly alkaline, especially the last. If urine be mixed with it, it is of course soon alkaline. In a mass of fæces the reaction at the surface may differ from that at the centre, and the changes on standing are very rapid. In typhoid or cholera they are alkaline, as a rule, in patients on a milk or starch diet they may be very acid.

Frequency.—By *diarrhœa* is meant frequent and fluid stools; by *constipation*, infrequent movements of the bowels, associated with symptoms which are relieved by purging. The normal stool is never fluid, but frequency is a variable matter and must be judged from the individual stand-point, hence subjective symptoms are necessary.

Diarrhœa may be due to increased peristalsis, increased intestinal secretion, or decreased absorption, and accompanies chronic enteritis, chronic peritonitis, intestinal tuberculosis, amyloid disease, cirrhosis of the liver, cholera, typhoid, dysentery, infectious diseases, uræmia, etc.

When the trouble is in the small intestine the movements are fluid and large but not necessarily very frequent; in dysentery they are frequent and scanty.

Constipation as a chronic condition is the result of careless habits of personal hygiene, of sedentary life, of a diet lacking in the constituents which stimulate intestinal peristalsis, of dilated stomach, constriction of the bowel, etc. Acute constipation occurs in obstruction of the intestine, paralysis of its wall as in peritonitis, and in meningitis and other conditions causing increased brain-pressure. In acute obstruction due to intussusception, ileus, etc., the frequent stools of bloody mucus but without fecal matter may deceive the doctor who does not personally inspect them.

The **consistency and form** of the normal stool vary considerably, depending on the habit and diet. Pathologically they depend on the intestinal secretion, absorption, and especially the motility. The stool may be abnormally too fluid or too solid; when very hard it is broken up into small masses resembling sheep manure, or somewhat larger masses, "scybala," which may be of even stony hardness and the size of a walnut. Such stools are common after typhoid fever and in some cases on a milk diet. These masses may in the rectum form large accumulations. When the mass is of very small caliber

[5] Zeitschr. f. klin. Med., 1903, Bd. 48, p. 163.

it does not necessarily mean a stricture of the lower bowel, since such is the form of stool in anal tenesmus, inanition, and certain nervous conditions. Boas emphasizes as occurring especially in stenosis of the lower intestine a stool which is homogeneous, thick, pasty or curd-like, and in which float short cylinders of formed stool about the thickness of the little finger. Such stools must be continuously present, however.

The fæces are abnormally soft: when they contain much fluid, which occurs when the motility is so rapid as to exclude absorption, when absorption is prevented, or when the intestinal secretion is increased as in cholera; when there is increased fat; a large amount of fruit or vegetable matter, especially cabbage, pear, apple, and plum; or, much mucus. A simple way of testing the difference between increased water and increased fat is to press the cover-glass down over a small portion on a slide. If on relieving the pressure the cover-glass stays, it is fat, while if it at once springs back and the air rushes in from all sides, it is due to water.

When stools are frothy it indicates an intense bacterial decomposition. In such cases they may also appear acholic, due to the changes in the pigment.

Color.—Normally the stools are dark in color, due to hydrobilirubin. The intestine is a reducing organ, hence bilirubin, except in a nursing-child, is never normally present.

The stools are darker the longer they remain in the intestine or are exposed to the air. In constipation they may even be tarry in appearance. The color also depends upon the food, being dark after a meat diet, light after milk; cocoa gives a reddish-brown color, wines a dark color, certain berries a greenish-brown, much chlorophyll a green color.

The color may depend on drugs: after doses of calomel they are sometimes green, due to biliverdin; after bismuth subnitrate a black color, due to bismuth suboxide; senna, santonin, gamboge, and rhubarb give a yellow color. Iron gives a dark brown, grayish, or even black color after the stool has stood in the air. This latter point is important, since a stool containing blood may resemble that containing iron, but the bloody stool is dark when fresh, the latter only after standing.

Clay-Colored Stools.—This may be the appearance of stools rich in fat, hence with the color of the bile hidden, but by extracting the fæces with alcohol and ether the presence of bile is proved; of diarrhœal stools which are pale since diluted; most important is the clay color due to the absence of bile, the true acholia, which gives the stools a grayish-white color with a bad odor, and soft from the abundance of fat; of those in which there has been active decompo-

sition with the resulting formation of the colorless products of bilirubin. In the last case the color is restored by reoxidation in the air or after calomel, and the stools are not putrid. This "leucourobilin" is as yet a rather hypothetical body. Such stools occur in a long list of conditions which have nothing in common.

Bilirubin is found in the intestine not below the lower ileum or ascending colon, except perhaps minute traces on masses of vegetable or of soaps and found only by such microscopic tests as Schmidt's. It is reduced in the large intestine to hydrobilirubin. Some of this is reabsorbed, hence more is present in a fluid diarrhœal stool than a solid, since it has escaped absorption. Bilirubin occurs in the stools of diarrhœa with increased peristalsis, in cases with disturbed absorption, or when the reduction is absent. The higher up the point of the disturbance the more of this pigment will be found, and yet the presence of bilirubin does not always mean trouble in the ileum, since some normally does reach the colon, hence may be present in the stools in colitis. Such a stool has an intense yellow or greenish color, and gives the Gmelin test beautifully. On the addition of one drop of yellow nitric acid the green ring of biliverdin can be at once seen. A case in which this test was most brilliant was one in Professor Müller's clinic, a case of catarrhal jaundice with clay-colored stools, who suddenly evacuated a large, soft, golden-yellow stool. One drop of nitric acid produced a most brilliant result. As explanation, it was supposed that an obstruction of the gall-ducts had been suddenly relieved, allowing the escape of a large amount of bile.

Schmidt's method is valuable to detect bilirubin. In a porcelain dish are mixed from 2 to 3 cc. of selected portions of the fresh stool, which portions are chosen to represent all the elements present, with concentrated aqueous mercuric chloride solution. This is ground up fine and allowed to stand twenty-four hours at least in a covered dish. Pure mercuric chloride should be used. The whole mixture should be acid. The fragments are then examined macroscopically and microscopically, those stained with hydrobilirubin turn red, those with bilirubin, green. Chlorophyll also will be green, and must be excluded microscopically. The biliverdin stage is never passed. Bilirubin occurs in adults only pathologically, and indicates usually an enteritis or intestinal catarrh, especially of the small but also the large intestine. The bilirubin is found most often on masses of cellulose, next on mucus, then muscle fibres and masses of fat, and lastly on the various other constituents. Since in the normal stool traces are not rare on cellulose fragments, it is the occurrence with mucus which is of greatest importance. This mucus may arise either in the small or large intestine. If in large glassy macroscopic masses, the origin is the colon; if of small macroscopic or of microscopic size,

the source may be the small intestine, especially if the stool be fluid. The source is surely the small intestine if the mucus contains many nuclei of cells the protoplasm of which is digested, or cells represented by fat droplets or bilirubin granules. The question is somewhat different in the case of bile-stained muscle-fibres or connective tissue and fat masses, since all in the small intestine are normally stained with bilirubin; hence their presence in the stool may mean trouble only in the colon, either too rapid peristalsis, or catarrh; to suggest trouble in the small intestine the above-mentioned masses of mucus also should be present.

Schlesinger [6] considers his test for urobilin (hydrobilirubin also) very delicate. The stool, if very fatty, is extracted with ether and then with acid alcohol. The reaction of the extract is made less acid with ammonia, an equal amount of zinc acetate solution (1 per cent. in absolute alcohol) added, and filtered. The filtrate gives a good fluorescence and spectrum.

Bile acids are normally reabsorbed, and hence do not appear in the stools.

Fatty Stools.—There is always some fat in the stools, providing there is much in the food. This may be as neutral fat, fatty acids, or soaps. The more difficultly melting neutral fats are present usually as white or yellow scales or droplets, according to their melting-point.

Fatty acids are usually in short, delicate, curved needles, and occur in thick masses, so that the shape of the individual crystal is often very difficult to make out. The soaps, on the other hand, occur in long needles which are arranged in clusters or fans, or in short plump crystals, or scales. The droplets of neutral fat are soluble in ether, the fatty acids are dissolved on warming and in ether, while the soaps are not dissolved on warming, nor are they soluble in ether unless they have been first split by acid. A test for neutral fat which is easily made is to mix the specimen under the microscope with one drop of concentrated alcoholic solution of Sudan III. which has been filtered just before using. The droplets take an orange to a blood-red color, while the soaps and the fatty acid crystals remain unstained. The students attempt to tell from crystal form alone whether it is soap or fatty acid crystals they see. A most instructive exercise is to give them for study two portions of the same stool, the one of which has been extracted with ether (but no acid added), and they see at once that the needle crystals are chiefly soaps.

Acholic stools usually contain much fat in crystals which are mixed homogeneously with the fecal matter. Such stools have a glistening gray appearance, and microscopically contain large num-

[6] Deutsch. med. Wochenschr., 1903, p. 561.

bers of fat droplets and large masses of fatty acid crystals which are very pretty to see.

In diarrhœa the masses of fat needles are present as minute points which may be seen with the naked eye.

Sometimes the clumps of fat are of a whitish-gray or a yellowish color like tallow, even the size of a nut; or the fat may be present as a melted oil which hardens over the stool or on the walls of the vessel containing it. The whole stool may resemble oil. In a recent case of supposed cancer of the pancreas the stool could not by appearance be distinguished from a mass of vaseline.

Such stools occur when there is an over-supply of fat ingested, hence especially in the olive oil treatment for gall-stones, in which case the lumps may vary from the size of a pea to that of a hazel-nut. A much smaller amount of fat may be conspicuous in small

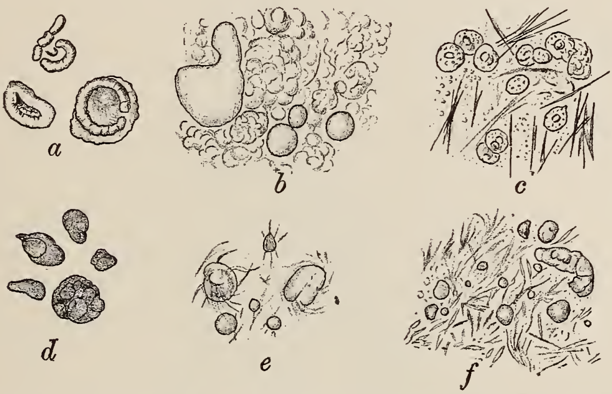


FIG. 65.—Forms of fats and soaps in stools (Schmidt and Strassburger). *a*, soaps; *b*, casein, and fat globules; *c*, fatty acid needles and leucocytes; *d*, yellow calcium soap; *e*, fatty acid crystals projecting from fat droplets; *f*, fatty acid and soap needles and scales from an acholic stool.

masses after a meal containing fats with a high melting point, as pork, mutton, or tallow.

Fatty stools are present when the mucosa or the lymphatics cannot absorb the fat: as in atrophy of the mucosa; amyloid disease; in all cases with extensive caseation of the retroperitoneal lymph glands, —tabes mesenterica,—the most common cause of fatty stools without jaundice, and in fact in a doubtful abdominal case the diagnosis of this condition is suggested by the stools alone; in peritonitis; and even in simple catarrh preventing absorption. There is a fat diarrhœa due to various diseases of the small intestine which should be distinguished from "diarrhœa pancreatica."

When bile is absent, from 55 to 78 per cent. of the fat will be in stools, instead of as normally from 6 to 10 per cent. Acholic stools in cases without jaundice are particularly interesting and may contain

large amounts of fat. The cause of this condition is problematical. Some consider that there is a cessation of bile secretion; others that in all such cases bile pigment was present but has been changed to the colorless forms.

In pancreatic disease fatty stools are common, yet to be of value the stool must be very fatty. Pancreatic disease without them occurs since fat is well used if already emulsified, although in such cases the fat is insufficiently split. Müller showed that while 84 per cent. of the fat

FIG. 66.—A sheaf of huge fatty acid crystals seen often in stools after they have stood a little time. $\times$ 400.

was normally split, if the pancreatic juice be excluded only about 40 per cent. Others have found more even 80 per cent. split, yet as fatty acid not as soap. The diagnosis of pancreatic disease is exceedingly difficult, it cannot be made from the fatty stool alone, and yet if all the elements of Le Nobel's symptom complex are present it should be easy;—no jaundice, glycosuria, much fat in the stools, many fatty acid crystals but no soaps, no hydrogen sulphide, skatol, indol, etc., the stools rancid but not putrid, and with few bacteria; if all these elements are present one is very safe in assuming some pancreatic trouble.

ESTIMATION OF FATS AND SOAPS.—The stool is first evaporated over the water-bath until of a semisolid consistency. It is then mixed with about 50 cc. of absolute alcohol, again evaporated, and this repeated until perfectly dry. Never try to dry down a stool without alcohol. A certain amount is powdered, dried at 100° C., and then weighed. It is then rubbed up with sand and extracted from eight to ten hours with ether. The ether residue is washed with warm water. It consists of neutral fats and the fatty acids. This is dried in a desiccator and weighed.

The neutral fat may be isolated and weighed by dissolving the residue again in ether and shaking it out with a dilute soda solution, which removes the fatty acids.

The amount of fatty acids is determined as follows: A weighed amount of the ether residue is dissolved in alcohol and ether and then titrated against alcoholic solution of potassium hydroxide, phenolphthalein used as indicator.

For the soaps, the fæces already extracted with ether are boiled with acid alcohol, dried, extracted again with ether, and the free fatty acid titrated. If one wishes to determine at once neutral fats and the split fats and soap, the stool is first boiled with acid alcohol and then extracted (Müller).

In fat determinations the following values are used:

(1) The *acid value*, that is, the number of milligrammes of potassium hydroxide necessary to neutralize the free fatty acid split from one gramme of fat. A weighed amount of fat is dissolved in alcohol and ether and titrated with tenth-normal KOH, phenolphthalein used as indicator.

(2) *Köttsdorfer's value*, the "saponifying value," that is, the number of milligrammes of potassium hydrate necessary to neutralize the fatty acid split off from one gramme of fat by saponification. A weighed amount of fat, 1 to 2 gms., is boiled with 10 cc. of half-normal KOH and 50 cc. of alcohol in a flask for a quarter of an hour on the water-bath, and titrated with half-normal acid, using phenolphthalein as indicator.

Hehner's value is the amount of fatty acid insoluble in water, which can be obtained by the saponification of 100 gms. of fat. One saponifies a weighed amount of fat, evaporates the alcohol, and treats the watery solution of the residue with hydrochloric acid. The free fatty acid is treated with boiling water, dried, and weighed.

The *Reichert-Meissl value*, that is, the number of cubic centimetres of tenth-normal potassium hydrate necessary to neutralize the volatile fatty acids obtained by the saponification of 5 gms. of fat. One saponifies a weighed amount of fat, acidulated with sulphuric acid, distils the volatile acid, and determines in the distillate the amount by titration with tenth-normal KOH (Thierfelder).

Mucus.—The stools always contain a certain amount of mucus (Boas). This is seldom seen even microscopically, and must be tested for chemically; any amount of visible mucus is somewhat abnormal. The mucus present is pure mucin, hence clouded by acetic acid. If rubbed up with water, an equal amount of lime water added, left to stand for several hours and then acetic acid be added, a cloud will indicate mucus.

Mucus is increased physiologically as the result of hypersecretion. This forms a glassy or cloudy coating over hard fecal masses, and evidently serves to protect the mucous membrane. It is poor in cells. It may also be present after an active purge.

That from the small intestine is intimately mixed with the stool and hard to isolate. In diarrhœa these small flecks can be picked out with a needle, and if the stool be solid they can be found as shreds or

lumps which never are bile stained. Such small flakes resemble gastric mucus. They are rich in cells and detritus of digestion, hence are not transparent. The bodies of the cells are often well digested. Some masses contain no cells but bilirubin granules and crystals in a cellular arrangement, as if the cells had been digested. The so-called "sago granules," or "spawn-like masses" of Virchow, Boas thinks are very rare. Mucus may be seen microscopically as small transparent lines and masses in the stool. The minute mucous granules or "islands" of yellowish or greenish mucus stained with bilirubin emphasized by Nothnagel as indicating catarrh of the small intestine Boas and Schmidt consider exceedingly rare, and for the most part albuminous matter rather than mucus. Yet small fragments are always present in acute enteritis. Much mucus is present in cancer of the rectum with stenosis. (See also page 390.)

If mucus be present in large amounts the stool may be jelly-like. In some cases it consists of mucus alone, is then glassy, jelly-like, thick, glistening in appearance, and means trouble no higher than the sigmoid. The white strips or tubes even several inches long, sometimes forming a cast of the bowel, seen in enteritis membranacea or colica mucosa, a secretory neurosis, are particularly interesting, indicating neurasthenia except in rare cases of pelvic tumor pressing upon the rectum. Membranous colic occurs mostly in women (80 to 90 per cent.), and in nearly all cases it is preceded by years of constipation. Some separate the cases into those with an inflammatory basis (enteritis membranacea), and those without, "colica mucosa," a pure secretory neurosis; others make no such division. Some patients have but one attack, some have one attack a day for even a week, and are then free for intervals of months. The masses of mucus are transparent, grayish-white or bloody, and are sometimes over a foot long. The movement consists of pure mucus without fecal matter. These strips of mucus are considered as tapeworms by the laity, or when large as pieces of bowel. Some persons will evacuate these at rather regular intervals. Their separation from the mucosa is often accompanied by very severe colicky pains. Their relation to intestinal sand is interesting, since in some cases these coexist and both may be due to a secretory neurosis (see page 371).

In searching the stool for small masses of mucus, vegetable masses, and especially fruit, must be excluded.

Blood.—It is of course necesary to exclude that from raw meat, and hemorrhage from the mouth, nose, lungs, and vagina. The blood may be suspected from the red or tarry black color of the stool, or found microscopically, or require chemical tests. The arrangement is important: fresh blood covering a formed stool indicates hemorrhoids; if evenly distributed with the food matter, it indicates hemor-

rhage in the stomach or the upper part of the small intestine, providing the stool is solid; if the stool be fluid it may be evenly distributed and indicate the small intestine, but usually the colon. In a liquid stool the color of the blood is a better criterion than is its arrangement, since the darkness in color increases in proportion to the height of its source. Tarry blood is seldom of low origin, while fluid blood usually comes from the colon or the rectum, and yet may be from much higher, in the small intestine as in typhoid fever, providing it be quickly enough expelled. In profuse gastric hemorrhage the stools may be of a black tarry color. In profuse typhoid hemorrhages, even when from some distance in the ileum, they may be bright red. From tuberculous ulcers there may be no blood. In cancer of the colon blood has been found in but 15 to 20 per cent. (?) of the cases. The bloody serous stools unmixed with fecal matter of volvulus and intussusception are particularly important. If the blood is fresh there is no difficulty in its recognition, but when there is doubt it is usually a hard question. The microscopical search for red blood-cells is unsatisfactory, since often no perfect cells are found, and all have broken down into masses of pigment, "hæmatoidin." The chemical tests are more satisfactory. Teichmann's acid-hæmin test does not always succeed, even in a sure case, since the right fragment may not have been selected.

The spectroscopic test is valuable to detect the hæmoglobin or hæmatin. Several cubic centimetres of the stool are mixed with water and a few drops of sulphuric acid until it reacts well to Congo red. It is then filtered and extracted with ether plus a few drops of alcohol. The ether solution is brownish-red from the acid hæmatin. In all cases the hæmatin from the meat must be excluded.

For the turpentine-guaiac test the stool is rubbed up with water and one-third volume of glacial acetic acid, and shaken out with ether. A few cubic centimetres of the extract are cleared by adding a little alcohol, then treated with ten drops of guaiac tincture plus thirty drops of turpentine. The blue color will indicate blood pigment. This test may be positive, however, if the patient has been eating potatoes and certain vegetables, or taking iron as medicine, or if there be much bile, saliva, milk or pus present. It is not reliable if but little blood is present, and when urobilin disturbs the reaction. (See page 366.)

The aloin test of Klinge and Shaer is more delicate, being positive after the ingestion of only 3 gms. of blood, hence more delicate even than the spectroscope; Joachim [7] advises to use both the guaiac and the aloin tests. Preliminary to this test Koziczowsky [8] advises

[7] Berl. klin. Wochenschr., 1904, xli. p. 466.
[8] Deutsch. med. Wochenschr., 1904, No. 33.

that all foods containing hæmoglobin and chlorophyll and all drugs be discontinued. The patient is put on a milk, bread, eggs, and fruit diet. Much fat is avoided and the diet period limited by charcoal, not carmine.

The stool if very dark in color is rubbed up with ten volumes of alcohol, and this filtered off to remove the urobilin. The stool is dried on the filter paper. About 5 gms. is digested one or two minutes with 5 cc. of glacial acetic acid, then all fat extracted with 10 cc. of ether. From 1 to 1.5 cc. of oxygenated turpentine are then superimposed and 0.5 cc. of fresh 3 per cent. aloin solution (0.3 gm. aloin powdered is dissolved in 10 cc. of 60 to 70 per cent. alcohol). At the line of separation is seen in from three to five minutes a fine red ring. This means the presence of blood if the patient has been on the above-mentioned diet for some days and if the test is positive on several examinations.

All fat must be removed; carmine will disturb the test, not so chlorophyll or urobilin; rare meats must be excluded, and all hæmoglobin-containing foods; all drugs containing iron. Of course, blood from the lung, mouth, and anal region must also be excluded.

The use of these tests on a large number of cases as a routine has developed some interesting results. In cancer of the stomach blood is practically always present in small amount in the stools; in ulcer of the stomach the blood is often present in larger amounts than in cancer, but not every day, there being blood-free intervals. In tuberculosis of the intestine there is none, in typhoid fever the test may be positive one day before the hemorrhage; in chronic passive congestion blood is usually present; also in mercury poisoning. In cirrhosis of the liver with venous stasis no blood was found. In some cases of hyperacidity of the stomach blood is present.

The test has the greatest value in the diagnosis of gastric cancer and the differential diagnosis of ulcer and nervous gastralgia.

Pus.—The presence of very much pure pus in the stools indicates the rupture of an abscess into the intestinal tract; in some cases it may be seen with the naked eye. The extent to which it is mixed with the fecal matter will indicate the height of its origin, and yet it is soon rendered unrecognizable by digestion and decomposition, so that even a large amount will not be suspected, as occurs in cases of appendix-abscess perforating into the intestine. The nuclei will remain visible for some time, but these cannot be distinguished from the cells of food. A small amount of pus, seen only microscopically, is often associated with mucus and blood. With blood it indicates intestinal disease, as catarrh or ulceration of the intestine, and in many cases cancer. Should the pus-cells be for the most part single, it is more in favor of catarrh, while if in small masses of even a

few, it indicates ulcer. It is not to be forgotten that from the normal mucosa leucocytes wander out and hence a few pus-cells may be expected. Casein curds and masses of albumin must be excluded.

Muscle and Albumin.—Muscle-fibres occur practically always in the stools. The more they are digested the less evident is their striation, so that while some will show beautiful cross striation, in others only the longitudinal striation is seen, and others can be recognized as muscle-fibres only from their shape, size, and color. They are nearly all bile-stained. They are increased on a rich meat diet, as in diabetes, in diarrhœa, in which case the masses may be visible to the naked eye, and where there is disturbed absorption or secretion. The question whether there is a pathological increase in a solid stool is best judged from their number and appearance (size, shape, striation) microscopically. One is soon able to form a pretty definite opinion.

This condition of *lientery* (the presence of grossly visible particles of undigested food) is, of course, seen best in cases with a gastro-intestinal anastomosis. It occurs in a variety of conditions.

The presence of an abnormal amount of muscle-fibre in a fairly thick or solid stool and without diarrhœa is known as azotorrhœa, which is suggestive but in no way conclusive in the diagnosis of pancreatic disease, unless diabetes also is present.

Curds of milk and masses of coagulated albumin—*e.g.*, coagulated egg—are seen as masses of amorphous granules forming yellow islands. The masses of milk curd are especially important in the infant stool. Soluble albumin, albumose, or peptone may be determined in the water extract by the ordinary tests. Normally they are present, but are increased in diarrhœa. If the biuret test be used, urobilin must be excluded.

Starch.—It is seldom that single well-preserved starch granules are seen in the stool of an adult, yet vegetable masses full of starch granules are common enough. If many well-preserved single starch granules occur it indicates some disturbance, either diarrhœa or hyperacidity. It is interesting that starch is never bile-stained. In the failure of pancreatic juice the starch is not increased, since the bacteria will break it up. Also the lack of bile causes no increase in the starch of the stool. The iodine test may be applied to indicate the extent to which the starch has been digested, a blue color indicating the unchanged granules; red, a slight digestion.

Carbohydrates.—To detect these Strassburger recommends the following: From 2 to 3 gms. of the dried stool (excluding mucus and lactose) are heated in a flask with 100 cc. of 2 per cent. HCl for an hour and a half (with a return cooler). It is then cooled and neutralized quite accurately with sodium hydroxide, filtered through

an asbestos filter, washed with water, and the filtrate brought to 200 cc. If necessary, it is filtered a second time. Fifty cc. of the filtrate are poured into a 300 cc. beaker and the sugar determined quantitatively. The amount determined of the grape-sugar multiplied by 0.94 equals the amount of starch originally present. Qualitatively, the stool may be boiled with water and the filtrate then tested with Trommer's or other solutions. It is best to precipitate the albumin with the acètate of lead; the lead is then removed with CO_2 and the filtrate tested. It is seldom, however, that any glucose is found unless the stool be first boiled with acid.

Ferments.—The stool may be extracted with glycerin and the digestive power of the extract tested; or, according to Leo, fibrin added, which will absorb the pepsin. The fæces are mixed with chloroform water until they form a thin pasty mass. In this is suspended from 2 to 5 gms. of finely divided, previously boiled blood fibrin enclosed in a gauze bag. In twenty-four hours this bag is removed, the fibrin washed a number of times with water, and then tested for the ferments which have been absorbed. To test for trypsin, a little of the fibrin is placed in a 1 per cent. solution of soda in an incubator and the biuret test applied to the filtrate at the end of a few hours. For diastase a little of the fibrin is placed in a thick starch solution in the thermostat, and in a few hours its filtrate tested with dilute Lugol's for the blue color of starch. Normally, these ferments seem destroyed or absorbed in the intestine, yet all may be present in diarrhœa.

Microscopy.—For the microscopical examination of the stools care must be taken in the selection of fragments, since the one who searches at random will often find nothing. In the case of parasite eggs, etc., it is best to mix the stool with water and allow it to sediment, or to centrifugalize it. Mucous particles are to be chosen if protozoans are the object of search. In searching for blood it often makes considerable difference whether the right particle is taken or not.

Epithelial Cells.—Squamous epithelial cells are often found in mucus which covers the stool, and come from the anal region; many are present in cases of rectal cancer and of proctitis.

Cylindrical epithelium is the commonest form found. For this the mucus should be studied, and especially that which is obtained by lavage of the rectum and sigmoid. These cells will show all grades of degeneration, from fairly well-preserved cells, even goblet-cells, to those which are very fatty, and finally those in which all trace of the nucleus is lost. They occur especially in diarrhœa, sometimes in such numbers that the term "desquamative catarrh" is applicable.

Triple phosphate **crystals** are almost always present, and are irregularly formed, as a rule. Calcium phosphate crystals occur in the same form as in the urine. In addition, are calcium salts of still unknown acids, which are present in irregular, oval, or circular

masses, sometimes fissured, sometimes with a concentric striation. These are always bile-stained. The calcium soaps and calcium oxalate are frequently found (see Fig. 65).

Cholesterin occurs often, but rarely in typical crystal form, and must be tested for chemically. Charcot-Leyden crystals have been found in a great variety of diseases, but it is the consensus of opinion now that their presence always indicates some animal parasite, although it may be any, from the harmless oxyuris to the pernicious uncinaria. They are, indeed, a very valuable indication when they occur in large numbers (see Fig. 67).

Bismuthous oxide occurs as black irregular rhombic crystals after the use of bismuth subnitrate (see Fig. 69). Hæmatoidin crystals occur, but are rare.

Remnants of undigested food form the chief part of the picture, especially the thorn-like spines from various fruits and berries; the

FIG. 67.—Charcot-Leyden crystals from the stools. × 400.

spiral cells, of which the veins of leaves are largely formed; the thick cellulose shell of various cells, some resembling soap masses, some parasite eggs; the elastic tissue from meats. The list is too long and varied to allow enumeration (see Figs. 69, 70).

MACROSCOPIC EXAMINATION. *Gall-Stones.*—It is often a matter of great importance to find these in cases of abdominal pain, and the stools should be carefully searched for at least fourteen days after a suspicious colic, since the most typical attack may be due to infection of gall-ducts without the presence of a stone. The stools are mixed well with water and then rubbed through a sieve. The gall-stones may not appear in the stool, since the softer ones certainly fall to pieces in the intestine, Naunyn thinking that only those with a hard rind reach the rectum. Also the stone may have been stopped in the duct without obstructing it. Their size may be even as great as a pigeon's egg. These stones consist of the calcium salt of bilirubin

and cholesterin, one or both. They may contain also biliverdin, bilihumin, bilicyanin, etc., and calcium carbonate in small amounts. Their consistency may be soft or very hard; they may show on fracture concentric layers. Some are smooth, facetted, others rough. If facettes are present, it means that more than one stone was present and that the source was probably the gall-bladder.

Pseudo gall-stones it is of great importance to recognize and to exclude. These may be woody plant masses, as the hard husks about the seeds of the pear. Such can be recognized on cross-section; they are harder than gall-stones. Fats, oils, and soaps of high melting point also form masses which may deceive. It is for this reason that the olive-oil treatment for gall-stones was formerly so popular, for it was followed by the passage of large numbers of pseudo-stones, soft,

FIG. 70.—Spines forming the "down," that on the right of a raspberry, on the left of a quince. These are often taken for the embryos of parasites.

fatty translucent masses very different on cross-section from the true stones.

Gall-sand—that is, very small stones (the size of sand grains), which sometimes appear in great numbers—may come from the gall-bladder, but this is rather doubtful, they probably being pseudo-concretions. True gall-sand would be dissolved in the intestine, nor would such an amount appear at one time (Naunyn).

The gall-stone is usually recognized from its fractured surface. This is necessary, since various intestinal concretions and contents (*e.g.*, a bird's vertebra) may closely resemble one. If necessary, it may be dried, powdered (an unpowdered stone will not dissolve since it is surrounded by a layer of mucus), and dissolved in alcohol and ether, which dissolves the cholesterin, which then will recrystallize out on evaporation; or the stone may be dissolved in boiling

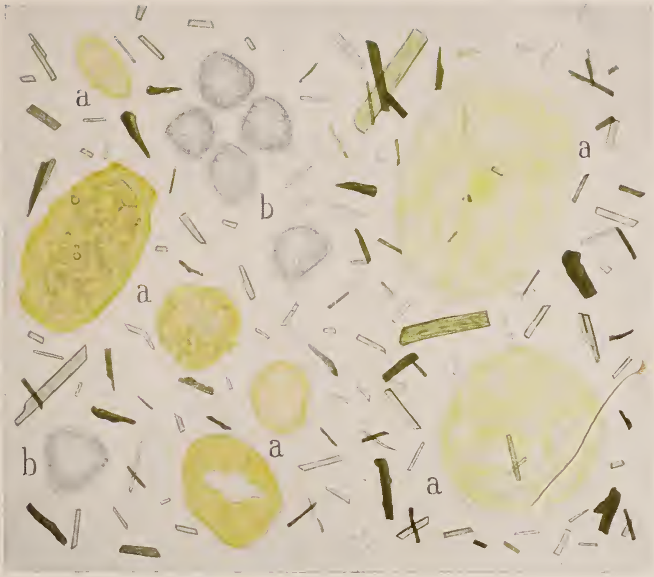


FIG. 68.—*a*, vegetable cells in stools, resembling parasite eggs; *b*, lycopodium spores; the crystals are an iron salt. × 400.

FIG. 69.—Cells in stools. *A*, *B*, muscle fibers; *C*, *D*, vegetable cells; *E*, *F*, spinal fibers from a piece of lettuce; *G*, cellulose framework of vegetable tissue. The crystals are of bismuthous oxide. × 400.

alcohol, in which case the cholesterin will precipitate at once on cooling. If to the residue after extraction of the cholesterin is added very dilute potassium hydroxide and cooled, a yellow solution of calcium salt of bilirubin will be obtained which will give Gmelin's test. If bilihumin be present it will give a blue.

Pancreatic stones are much rarer than gall-stones. They are white in color, and consist chiefly of calcium carbonate, hence will effervesce on the addition of hydrochloric acid.

Enteroliths.—By these are meant incrustations of food masses with inorganic salts. Their greatest importance is, of course, in appendicitis. They seldom occur in the fæces. The salt present is for the most part triple phosphate.

Intestinal Sand.—Intestinal sand is a very interesting find. By this is meant small granules of inorganic salts, the phosphates and carbonates of calcium especially, but also of magnesium, iron, and other metals. There is also a certain amount of organic matter, also fat, a good many bacteria, no cholesterin, sometimes urobilin. They are spherical or irregular, very hard, about the size of the head of a pin, 0.15 to 2.5 mm. in diameter, often of a reddish-brown or green color, and may be found in the stools at certain times in large numbers, even half an ounce at once. Most of the cases thus described are of pseudo-sand. Some granules seem of blood pigment, some of bile pigment, others of drugs taken (*e.g.*, salol). Some have as nucleus a grain of quartz sand swallowed and coated with phosphates; others are the seeds of various berries, or the small hard granules from the seed-case of some pear. These can be easily excluded by microscopic examination of the cross-section. True intestinal sand, of which we have seen but one good case, seems to be the result of a secretory neurosis of the intestine. It occurs in neurasthenic persons, and often in association with membranous colitis. Such stools are often preceded by about an hour of severe pain. Eichorst refers to it as a "gravel-forming enteritis," perhaps caused by bacteria. The large number of bacteria and the reduced bile pigment in some indicate the large bowel as the place of formation, although the absence of the latter makes Thompson and Ferguson think it in their case formed in the ileum. The granules also may, as in our case, appear in large amounts at certain intervals not related to diet, with nervous symptoms, and without mucus. Calcium sulphate has been found to be the chief constituent of some.[9]

Bedford[10] thinks his case shows a relationship to gout and tophus formation.

[9] See also Garrod, Lancet, March 8, 1902, and Eichorst, Deut. Arch. f. kl. Med., 1900, Bd. 68, page 1.

[10] Lancet, July 26, 1902.

Tumor Fragments.—Tumor fragments and adenomatous polyps, which may occur as an independent disease or grow in the neighborhood of cancers or ulcers, may appear, having their origin in the rectum, colon, or even higher. They are hard to recognize. If the stool is thin, these firm fragments of a grayish-red color and firm consistency may be found and the diagnosis made from the arrangement of the nuclei in the sections; but the fine details will all have been lost.

Intestinal Parasites. Protozoa. Rhizopoda.

AMŒBA COLI.—This amœba (see Figs. 71, 72) is now generally granted to be the cause of the so-called amœbic dysentery; the bacillary dysentery is a different disease. In a well-marked case of amœbic dysentery the stools contain much blood-stained mucus containing large numbers of these parasites. It is of the utmost importance that the stool be examined while fresh and warm, since the parasite is

FIG. 71.—Amœba coli (Entamœba dysenteriæ), common form. × 400.

very susceptible to cold and to an acid reaction of the medium, hence soon becomes unrecognizable.

Although dysentery is usually present, it is not always, and the amœbæ may be found in the hard constipated fæces of cases admitted during remissions of the disease, for liver abscesses, *e.g.* It is much better to examine the mucus removed by a rectal tube than the stool, and a small fleck in the eye of the tube is enough. In any case mucus, especially blood-stained, should be searched for, and if none found the liquid part of the stool should be examined. It is desirable to use a warm stage, although we find the top of the steam radiators very satisfactory. Since many degenerated epithelial cells and cells from the food look very much like amœbæ, the rule should be inviolable to always see definite amœboid motion, that is, the extrusion of true pseudopods not merely a change of shape, before pronouncing the object an amœba. This rule is hard to follow, especially

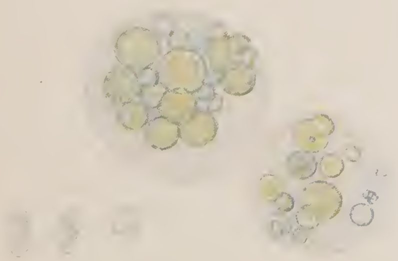


FIG. 72.—Amœba coli (Entamœba dysenteriæ). An uncommon, very hyaline, and very amœboid form of parasites, usually filled with red blood-cells. The small forms are true amœbæ from a normal case, Entamœba coli, drawn to the same scale. × 400.

if amœba-like bodies full of red blood-cells are found in blood-stained mucus.

Biologists may be able to recognize amœbæ under all circumstances; not so the clinician. In the intestines occur so many cells which resemble resting amœbæ, and degenerating cells may show such lively change of shape especially on the warm stage, that it is much safer to examine the fresh warm stool, and in case no amœbæ concerning which there is no doubt, with distinct extension of a hyaline ectoplasm from a granular endoplasm, and definite wavy motion of this pseudopod, then its retraction, or better a definite progression by means of it, be seen, it is much safer for the patient if one looks further for the diagnosis. Of course, the reply may be made that these "degenerating epithelium cells" may in truth be the harmless form of amœbæ, but as these and dead cells are said to have equal pathogenic importance, the mistake does little harm.

Amœba coli is from 8 to 50 microns in diameter. It consists of an endosarc which is more or less finely granular and which may contain leucocytes, red blood-cells, bacteria, epithelial cells, and particles of food which the parasite has ingested, and a clear hyaline ectosarc best seen in the pseudopods. These pseudopods may be projected and revolve around the parasite without causing any change in position, or by means of them the parasite may move, even so fast on a well-warmed stage that it is difficult to follow it; or the parasite may not move, but change its shape repeatedly by projecting one, two, or even more pseudopods in various directions. The nucleus is best seen in the parasite just dead or which has been killed by acetic acid or corrosive sublimate. It is spherical and about 6 microns in diameter. As a rule, it is not clearly seen in the living parasite, and yet our cases differ in this particular. In some nearly every amœba will have a very distinct nucleus, whereas in others hardly one can be well made out, the endosarc of the parasites being filled with detritus. In the endosarc may also be seen one or several vacuoles which do not pulsate. In fact, the parasites in different cases may vary so markedly in appearance, in size, etc., that one is always tempted to classify them in several groups on this basis.

Resting forms occur in which, it is stated, the nucleus divides repeatedly, and this is supposed to be the form which infects another host.

Since amœbæ are found in conditions without ulceration of the intestine, in diarrhœa, typhoid, acute and chronic enteritis, colitis and proctitis, and in the stools of normal men, some doubt the pathogenicity of the parasite, while many separate the pathogenic Amœba coli from a harmless form. Quincke and Ross separated:

Amœba coli (Lösch), 15 to 25 microns in diameter; encysted forms, 10 to 15 microns, pathogenic to man and to cats.

Amœba coli mitis, 25 to 35 microns in diameter, slightly patho-

genic to man causing a mild enteritis, and not at all to cats. This form never contains red blood-cells, but many bacteria and other food elements.

Amœba intestini vulgaris, similar to the "mitis" but not at all pathogenic.

True, sometimes are seen small amœbæ, less than half the size of the ordinary Amœba coli, in cases of diarrhœa, with large numbers of flagellates. In several recent cases these small active amœbæ were present in large numbers. They contained no red blood-cells, the stools contained neither mucus nor blood. Large numbers of Lamblia, Cercomonas, and Trichomonas were also present.

The best recent contribution to the subject is by Schaudinn,[11] who separates Entamœba coli from Entamœba histolytica,[12] the former the common harmless variety, the latter the pathogenic form causing dysentery.

Entamœba coli can be found in the stools of 65 per cent. of normal persons after a dose of Epsom salt (Craig). Its size averages somewhat smaller (10 to 20 microns in diameter), it is less actively motile, there is less difference between endosarc and ectosarc, the latter is less refractile, the former has less demonstrable structure, vacuoles are less common, and the nucleus more distinct than in the pathogenic variety. What is more important, the pathogenic variety shows no encysted stage, but does multiply by sporulation. (For more details, see Craig, loc. cit.)

Flagellata.—In human parasitology the flagellata which are important are of the enflagellata, and of these the protomonadina and the polymastigina. Flagellated rhizopods and lower plants must be excluded as extraneous.

Polymastigina.—These are flagellata with three equal or from four to eight unequal flagella inserted at different points. They may also have an undulating membrane, often mistaken for a row of cilia. Of these are two groups of importance, the Trichomonas and Lamblia.

TRICHOMONAS.—This is a pear-shaped organism, rounded in front, pointed behind, with at its anterior end three to four equally long flagella which often are united at their base. The undulating membrane, which is usually present but not always seen, begins at the anterior pole and extends obliquely backward. The nucleus is anterior, and behind it are one or more vacuoles which do not pulsate. It is interesting to study the various sizes and shapes of these flagellated organisms, and their movements, particularly so when in an old specimen the flagella have been withdrawn and then evidently the attempt made to extrude them, in which case the membrane is

[11] Arbeit. a. d. K. Gesundheitsamte, 1903, xix, p. 563.

[12] Craig, Am. Med., May 27, June 3, 1905, considers the name Entamœba dysenteriæ better.

FIG. 73.—Parasite eggs in stools. *a*, *b*, *c*, eggs of trichocephalus dispar, showing the different colors (species ?); *d*, *e*, ascaris lumbricoides: *d*, envelope lost; *e*, perfect. $\times$ 400.

projected to some distance in three or four different directions resembling the struggles of a cat which is tied in a bag.

Trichomonas Vaginalis (Donné).—This parasite (Fig. 74) is from 15 to 25 microns long, from 7 to 12 broad, with its posterior end drawn to a thread, its cuticle thin, protoplasm free from granules. It has three flagella, as a rule, which sometimes seem united at base, the fourth, which is sometimes described, probably being the edge of the undulating membrane. These are of equal length. The undulating membrane extends spirally backward from the anterior pole. This parasite is found in abundance in the acid secretion of catarrhal vaginitis.

In the intestine various forms have been described under such names as Protoryxomyces coprinarius, Monocercomonas hominis (Grassi), Cimænomonas hominis (Grassi), Trichomonas hominis (Grassi), Cercomonas coli hominis (May), but all of these are now

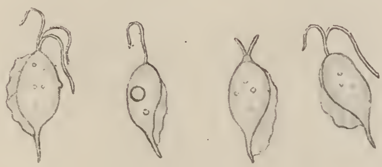


Fig. 74.—Trichomonas vaginalis.

considered to be the same as the above-mentioned Trichomonas vaginalis, which parasite can live in the vagina, the urethra, the large and the small intestine, the stomach, even appear in the mouth, and be found in the sputum from lung cavities and in the Dietrich's plugs. It has long been a question whether these parasites were harmless or not; whether they caused a diarrhœa or merely aggravated a trouble that was already present. It is now considered by many, and we could mention one or two cases in this connection, in which this parasite seems to have been the cause of a severe diarrhœa.

Lamblia (Fig. 75).—This is a family of pear-shaped organisms with a deep concavity on their inferior surface and with four pairs of flagella, three on the edges of the concavity and one at its posterior extremity. Various names of this parasite are Lamblia intestinalis, Cercomonas intestinalis (Lambl), Cercomonas coli (May), Trichomonas intestinalis (Leuckart).

The protoplasm is hyaline and finely granular, never containing solid inclusions, and with a very fine cell membrane. The nucleus is dumb-bell-shaped and at the base of the concavity. It has four pairs

of flagella of almost equal length (9 to 14 microns), one on each side of the concavity, two pairs at the projection at the inferior edge of the concavity, and one pair at the end. This parasite lives in the jejunum and the duodenum, and sits still on the top of a columnar cell, which it embraces with its concavity. In some cases they are found in such numbers that they form a membrane covering the mucosa. When they reach the large intestine they are encysted, and then are round or oval bodies with a very distinct membrane, within which is the folded organism. The motile parasite is thus not seen in the stools unless in a severe diarrhœa, in which case they have not had time to encyst themselves. They then move with some rapidity and very irregularly, lashing about in an aimless manner. They vary from 10 to 21 microns in length, and from 5 to 12 in width. The

FIG. 75.—Lamblia intestinalis, showing the motile form in different positions, and stages of its encysting. × 900.

encysted forms, from 10 to 14 microns long by 8 to 10 wide. The stools should be examined as fresh as possible and on a warmed stage. The number in the stools may be enormous, even estimated at eighteen millions in twenty-four hours. Their surest point in diagnosis is the concavity and the dumb-bell-shaped nucleus. The host is chiefly the mouse, rat, rabbit, dog, sheep, cat, etc. Men are evidently infected from water. They have been found principally in children. While their pathogenicity is uncertain, they may aid in the disease, and they certainly live best where there is intestinal trouble.

We have recently had a case of marked infection in a medical ward, recognized in the stool by the encysted forms. When purged with Epsom salt the motile Lamblia was easily found. The egg-like encysted forms were present in the fatty stools in great numbers, from five to ten being present in most of the fields (400 x). It was

interesting to watch the organism encyst itself, first withdrawing its tail flagella, then becoming more oval, with the concavity last to disappear, from the edges of which the flagella projected until the cavity disappeared. In some cases the lines in the encysted form, commonly taken to indicate the folds of the parasite, seemed the edges of this closed cavity. A membrane could in some be distinctly seen.

Protomonadina, forms which have one or two equal or one principal flagellum and one or two smaller ones, are much smaller and of a lower class than the above mentioned polymastigina. Two of the three forms occur in man, Cercomonadidæ, which have one flagellum and no undulating membrane, and the Trypanosomidæ which have one flagellum and an undulating membrane which reaches the whole length of the parasite.

CERCOMONAS HOMINIS.—These parasites are small flagellates occurring in the stools, from 10 to 12 microns in length, but varying from 8 to 16 microns. They are pear-shaped, with a long flagellum at the anterior end which may be even twice the body-length. They move very rapidly. Their pathogenicity is doubted. They have also been found in other parts of the body, including the sputum.

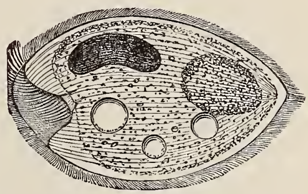


FIG. 76.—Balantidium coli. (Copied from Braun.)

Infusoria.—The infusoria are bilaterally symmetrical protozoans which have a permanent shape, are ciliated, contain contractile vacuoles, and usually a macro- and micro-nucleus. The order which is of most importance to us now is that of Heterotricha, which are uniformly ciliated, but with a border of longer cilia around the peristome, and of these, the Balantidium group.

BALANTIDIUM COLI OR PARAMÆCIUM COLI.—These parasites (Fig. 76) are oval, covered uniformly with cilia, are from 60 to 100 microns long, from 50 to 70 broad, with the mouth at the anterior end, a funnel or cleft-shaped entrance extending one-fourth the body length and surrounded by cilia about twice as long as those over the body. The ectosarc and the endosarc are clearly differentiated. The latter is finely granular, containing many fat or mucous droplets, starch granules, even red blood-corpuscles, leucocytes, and bacteria. The nucleus is kidney- or bean-shaped and also accompanied by one or more accessory nuclei. Usually there are two contractile vacuoles which pulsate feebly. The surface is traversed by parallel longitudinal lines connecting the two poles, most distinct at the anterior end. The anal orifice is at its posterior end, which is rather blunter than the anterior. This parasite occurs especially in the colon, but in severe cases may be found even in the jejunum, and may be present

in the stools in some cases in enormous numbers, even from one to two hundred in one drop. The blood-stained mucus should be examined, which also contains many epithelial cells. The pathogenicity of these parasites has been questioned, and yet it is now quite unanimously granted that they may be the cause of the most severe and stubborn catarrh, which may even be fatal. Others say they invade a catarrhal intestine as secondary parasites; others say they may set up a catarrh which continues after they die out (Henschen). Between eighty and ninety such cases are now on record, especially from Russia. A very good description is that given by Strong and Musgrave.[13] Klimenko[14] concludes that the diarrhœa may first be due to their mechanical irritation of the rectal mucosa, later to catarrhal or even ulcerative colitis; that they invade the intestinal wall, enter the blood-vessels, and sometimes cause emboli to distant organs; but that their action and effect are chiefly mechanical is shown by the absence of any degenerative or inflammatory changes which would point to a toxine.[15]

We have seen these parasites in a few cases of diarrhœa, but in none severe enough or in numbers great enough to attach much importance to their presence.

Enthelmintha. Trichina Spiralis.—The adults or the embryos may be in the stools, but they are rarely found. Adults occur in intestines of rats, pigs, dogs, and cats. The male worm is from 1.4 to 1.6 mm. long and 0.04 mm. wide, the female 3 to 4 mm. long by 0.06 mm. wide. After the encysted embryos are swallowed with meat the capsule is digested in the stomach and the embryos rapidly mature in the intestine. On the second day the males die and the females bore their way into the mucosa of the villi, or at the base of the Lieberkuhn glands, at which point they lie in the lymph spaces and viviparously hatch the young (0.09 to 0.1 mm. long, 6 microns wide) into the lymph and blood-stream. These in nine or ten days begin to take their permanent habitat in the muscles, travelling passively in the blood-stream and also actively boring their way. They are then about 1 mm. long. A capsule is formed around them, and in about one year this begins to calcify.

Ascaris lumbricoides.—This, the ordinary "round worm," is a common intestinal parasite, occurring in about 0.4 per cent. of all cases (Garrison, Ransom, and Stevenson). The female is from 20 to 40 cm. long, 5 mm. thick, the tail straight and conical. The male is from 15 to 25 cm. long and 3 mm. thick. The posterior end is bent ventrally into a hook, and terminates in the two spicules. The mouth of both is surrounded by three papillæ. The color of these worms is gray or a dirty reddish-brown. While it is an inhabitant of the small intestine, and hence is most commonly seen in the stools,

[13] Johns Hopkins Hosp. Bull., February, 1901.
[14] Beitr. z. path. Anat. u. allg. Path., 1903, Bd. 33, p. 281.
[15] Ehrnrooth, Zeitschr. f. klin. Med., 1903, vol. xlix. p. 321.

yet it is often present in the vomitus. Its eggs (Fig. 75, *d, c*) are found in the stools in large numbers. These are elliptical, 50 to 70 microns long and 40 to 50 microns wide. Those which we have measured varied from 65 to 80 by 45 to 55 microns, including the envelope, and 59 to 72 by 40 to 50 microns not including this. These eggs

FIG. 77.—Oxyuris vermicularis. A, B, and C, adults; A and C are females full of eggs. × 12. D, egg, × 400.

have an unsegmented protoplasm surrounded by a thick transparent shell, which in turn is covered by a thick, gelatinous very uneven lumpy envelope, which is usually bile-stained. This envelope may be lost, in which case the smooth-shelled egg has been mistaken for the uncinaria. In the diagnosis of this worm it is to be recommended

that santonin be given, which will have a therapeutic as well as a diagnostic value. These worms occur in children especially. Few are present, as a rule, although very many in some cases.

Smith and Goeth [16] consider the worm they report a new species, —"Ascaris texana."

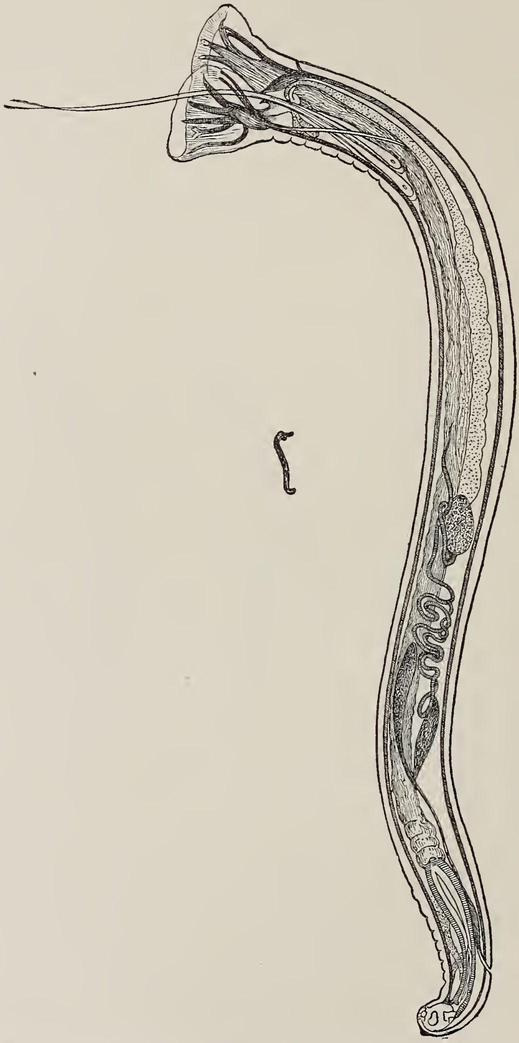


Fig. 78.—Ankylostomum duodenale, natural size to left, much magnified bursa of male on right. (From Braun.)

Oxyuris Vermicularis.—This little parasite (Fig. 77) occurs in the rectum and colon even as high as the cæcum where it inhabits the appendix, but it may even reach the stomach. It can travel

[16] Jour. Am. Med. Assoc., 1904, No. 8.

through uterus and tube to Douglas's cul-de-sac. According to some, it can bore its way through the intestinal wall and cause an abscess. It is present in perhaps 0.8 per cent. of adults. The adult male is from 3 to 5 mm. long, with its posterior end bent into a ventral hook. The female is 10 mm. long and 0.06 mm. wide. They are white in color. Their eggs are 50 microns long and 16 to 20 microns wide, and have a characteristic asymmetry. The parasite leaves the rectum to lay its eggs on the skin surrounding the anus, at which time the itching occurs. The eggs when deposited already contain a well-developed embryo. It is rare to find the eggs in the stools, except in the mucus which the stool gains on passing through the lower rectum, hence the skin around the anus should be

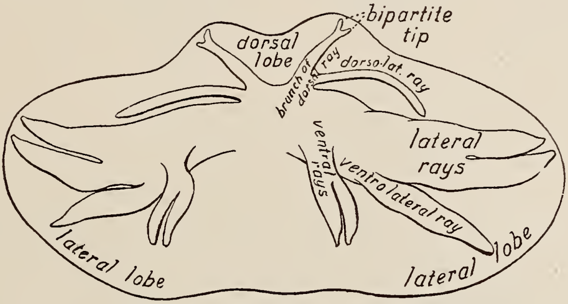


Fig. 79.—Caudal bursa of Uncinaria americana. (Schematic.)

examined for the adults. It is only accidentally that eggs are found except by scraping the surface epithelium from the margin of the anus, and this is the best method.

Very good plates of the development of this worm are given by Heller.[17]

Uncinaria Duodenalis and Uncinaria Americana.—These two parasites (Figs. 78-84), belonging to the nematode family, Strongyloidæ, are the cause of some of our severest anæmias. They have recently attained great importance in this country through the demonstration by Stiles that they are the common cause of the "anæmia of the South." In five hundred cases chosen at random they were present in 3 per cent.[18] They have long been known in their connection with bricklayer's anæmia, tunnel-workers' anæmia, Egyptian chlorosis, miners' anæmia, etc. The best description of these parasites is that given by Stiles in the Eighteenth Annual Report of the Bureau of Animal Industry, 1901.

Uncinaria Duodenalis, Ankylostomum Duodenale.—The body is cylindrical, somewhat attenuated anteriorly. The buccal

[17] Deutsch. Arch. f. klin. Med., 1903, Bd. 77, p. 21.
[18] See also Smith, Am. Jour. Med. Sci., 1903, vol. cxxvi.

cavity (Fig. 82) has two pairs of ventral teeth curved like a hook and one pair of dorsal teeth directed forward; the dorsal rib does not project into the cavity. The male is from 8 to 11 mm. long with a caudal bursa (Fig. 80) with dorso-median lobe, and prominent lateral lobes united by a ventral lobe. The dorsal ray divides at a point

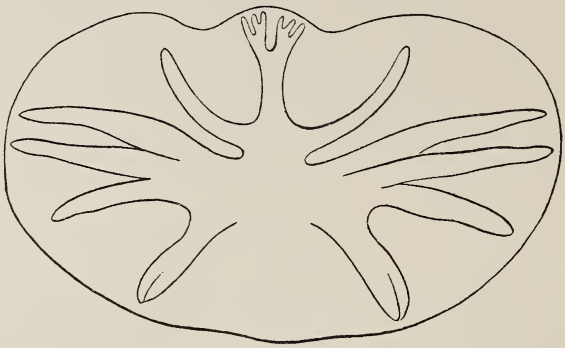


FIG. 80.—Caudal bursa of Uncinaria duodenalis. (Schematic.)

two-thirds its length from the base, each branch being tridigitate. The spicules are long and slender. The female is from 10 to 18 mm. long, the vulva at or near the posterior third of the body. The eggs are ellipsoid, 52 by 33 microns, laid in segmentation. Development is direct without intermediate host.

UNCINARIA AMERICANA (Stiles, 1902).—This differs from the

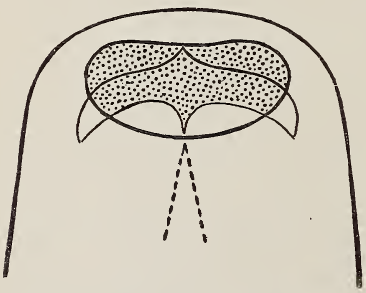


FIG. 81.—Head of Uncinaria americana. (Schematic.)

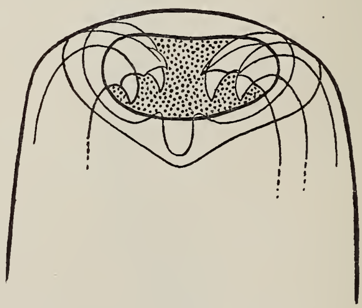


FIG. 82.—Head of Uncinaria duodenalis. (Schematic.)

above-mentioned form in that its buccal cavity (Fig. 81) has a dorsal pair of prominent semilunar plates or lips, and a ventral pair of slightly developed lips of the same nature, no hook-like teeth. The dorsal conical median tooth projects prominently into the buccal cavity. The male is from 7 to 9 mm. long, the caudal bursa (Fig. 79) with a short dorso-median lobe, which often appears as if divided into two lobes, and with prominent lateral lobes united

laterally by an indistinct ventral lobe. The common base of the dorsal and dorso-lateral rays is very short. The dorsal ray is divided to its base, its two branches being prominently divergent and their tips bipartite. The spicules are long and slender. The female is 9 to 11 mm. long, the vulva in the anterior half of the body but near the equator. The eggs are ellipsoid, 64 to 72 by 36 to 40 microns,

Fig. 83.—Eggs of Uncinaria duodenalis. *a*, unsegmented; *b*, with four segments and showing nuclear spindles; *c* and *d*, later stages of segmentation. × 400.

in some cases partially segmented in utero, in other cases containing a fully developed embryo when laid.

The eggs (Fig. 83) of the Uncinaria worms are found in the stools either unsegmented or during the early stages of segmentation. They have a thin clear shell. While the yolk will show all stages of segmentation, it is rare to find eggs with an undivided yolk, those

Fig. 84.—Embryo of Uncinaria (americana?) found in the stool. × 400.

divided into four, eight, sixteen, or more segments being the most common. The eggs should be searched for in the fæces, a small amount being mixed in a drop of water and spread on the slide. The older the fæces and the warmer the weather the more advanced will

the segmentation be. Of Uncinaria americana it is more common to find the fully developed embryo within the egg-shell or free.

It is well in a suspected case to allow the stool well mixed with water to sediment, or to centrifugalize it; the eggs will be found at the bottom. The adults may be found in the sedimented stool after a small dose of thymol followed by oil. The adults are usually red from the blood with which they are filled. They occur in the duodenum, jejunum, and ileum, many thousands sometimes in one person, although, as a rule, not more than a few hundred. While they do not multiply in the intestine they may live there for five years. They cause a severe chlorotic anæmia.

Stiles suggested the simple test of placing a small portion of stool on white blotting-paper and allowing it to stay for an hour or longer. On removing it a reddish-brown stain remains.

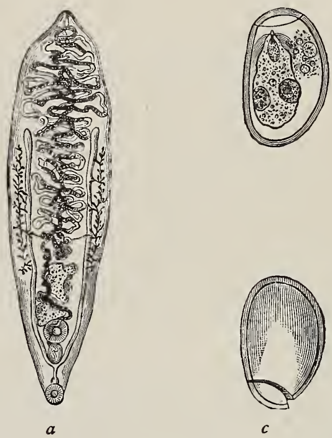


FIG. 85.—Distoma lanceolatum. *a*, adult; *b*, egg with embryo; *c*, empty shell. (From v. Jaksch.)

TRICHOCEPHALUS TRICHIURIS. TRICHOCEPHALUS DISPAR. TRICHIURIS TRICHIURA.—This is the ordinary whip-worm, a worm from 4 to 5 cm. long, with two-thirds of the length a whip-like tail. They occur especially in the cæcum, but also in the colon; rarely in the small intestine. They are perhaps one of the most common of intestinal parasites, in 10.3 per cent. of adults in this country, but in the stools of 45 per cent. in some parts of Germany, and at autopsy in 100 per cent. in Southern Italy. Their eggs (Fig. 73) are very characteristic, being from 50 to 54 microns long and 23 microns wide, with an unsegmented yolk and a very thick shell, into each pole of which is inserted a plug. These eggs present an interesting variety of colors, some being light lemon-yellow, some deep yellow and some a dark brown. In the stools we have also found eggs which were certainly those of this worm, but which had no

plugs at the end. These may be very young eggs, since they have this shape. This parasite is harmless as a rule, but may cause enteritis and the severest and even fatal anæmia. In a recent review of the effect of this worm, Becker [19] classifies the symptoms as gastro-intestinal, diarrhœa due to ulcers or catarrh, blood in the stools, symptoms of appendicitis even; nervous symptoms simulating meningitis (Erin thought beriberi due sometimes to this worm); and anæmia with all its symptoms.

Strongyloides Intestinalis.—Anguillula stercoralis et intestinalis; Leptodera stercoralis et intestinalis; Rhabditis stercoralis, Rhabdomena strongyloides, are a few of the many synonyms. The rhabditiform larvæ of this parasite found in the stools measure from 0.3 to 0.6 mm. long and from 16 to 22 microns wide. They are in very active motion. The best way to find them is to make a depression in the fecal mass, fill it with water, place the stool then in a thermostat,

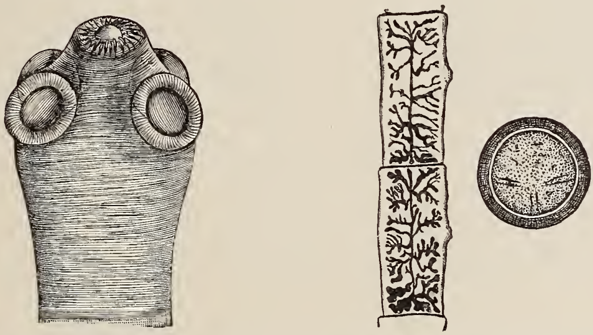


Fig. 86.—Tænia solium: Head (magnified), proglottis (actual size), and egg (magnified). (Zeiss's eye-piece IV., objective IV.) (From a preparation by Cori and v. Jaksch.)

and examine a drop of this water the next day for the eel-like worms. The eggs do occur, but very rarely, and could hardly be distinguished from those of Uncinaria duodenalis, but they are perhaps a little larger, measuring from 65 to 70 microns long and from 34 to 39 microns wide, and are very much segmented. In the intestines all stages of the development of the embryo may be followed.

The adult female resembles a filaria; it measures from 2.1 to 2.2 mm. long and 32 to 39 microns wide. The body increases slightly and gradually in size from the head to the posterior quarter, and then terminates rather suddenly in a short tail. The male is about one-fifth smaller.

The worms are abundant in the duodenum, fewer in the jejunum. The adults are found very rarely in the stools. They occur in about 0.6 per cent. of all persons.

[19] Deutsch. med. Wochenschr., 1902, June 26.

Trematodes.—Eggs of Distoma lanceolatum and Distoma hepaticum have been found, but rarely, in the stools. Those of Distoma lanceolatum are yellowish when young, dark brown when older, from 38 to 45 by 22 to 30 microns in size. They have a thick shell with a lid, and contain a ciliated embryo. Those of Distoma hepaticum (Fasciola hepatica) are yellowish-brown, oval in shape, and from 130 to 145 microns long by from 70 to 90 microns wide (see Fig. 85).

Cestodes.—In a suspected case of tape-worm it is always important that segments be seen before the treatment, which is severe, is undertaken. We have had sent to this clinical laboratory, for instance, mucous casts of the intestine under the supposition that they were decomposing tape-worms. Certain food constituents are also thus interpreted. To determine the success of treatment the head should be searched for; the stool is well mixed with water and allowed to settle for ten minutes, and then the upper fluid decanted; this is repeated several times; the heavy head will settle to the bottom. If the head is not found a cure is not certain till three months have passed without the reappearance of segments.

TÆNIA SOLIUM.—The infection is derived from Cysticercus cellulosæ of pork. The adult worms in the intestine average about 3 m. long, although much longer have been described; the head varies from 0.6 to 1 mm. in diameter, with four suckers from 0.4 to 0.5 mm. in diameter, and a rostellum with a double crown of 22 to 32 hooks from 0.11 to 0.18 mm. long. The neck is about 3 cm. long, and is unsegmented. The ripe segments are from 9 to 10 mm. long, by from 4 to 5 mm. wide. The genital openings are at the margin and arranged in a fairly

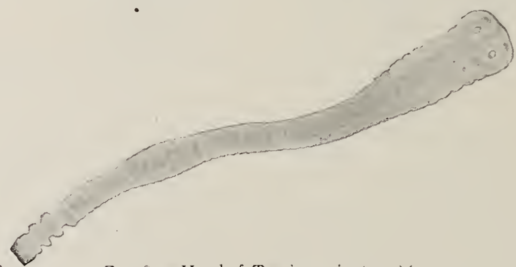


FIG. 87.—Head of Tænia saginata. × 5.

regular alternating manner. The uterus is characteristic, consisting of a large median stem and on each side from seven to ten coarse branches, each one of which branches dendritically. The eggs are round or oval, the shell very thin but surrounded by an embryonic shell which is thick, with radiating lines, and often yellow in color. These eggs are about 35 microns in diameter. This worm is excessively rare in America, if, indeed, any cases have been found, those exhibited in museums being for the most part cases of mistaken diagnosis (Fig. 86).

TÆNIA SAGINATA.—The beef tape-worm, the infection arising from the cysticercus of beef and, perhaps, of sheep, is in this country quite common. The adult worm varies from 4 to 8 m. or more in length. The head (Fig. 87) is from 1.5 to 2 mm. in diameter,

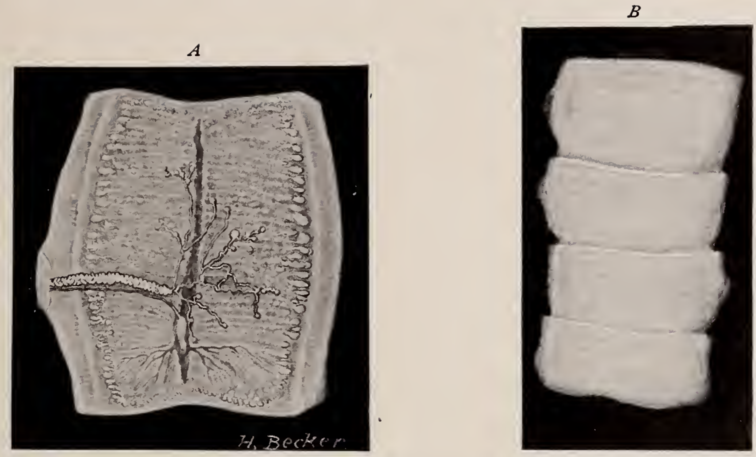


FIG. 88.—*A*, ripe link of Tænia saginata. × 3. *B*, four unripe links. × 3.

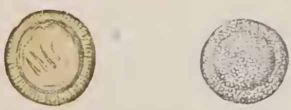


FIG. 89.—Eggs of Tænia saginata × 400.

cuboid in shape, with four suckers each 0.8 mm. in diameter, and without hooks. The neck is long and delicate. The ripe segments (Fig. 88) are from 16 to 20 mm. long by from 5 to 7 mm. wide. The over-ripe segments are longer and somewhat more slender. The genital openings are at the margins and irregularly alternate. The uterus is characterized by the multitude of its fine branches, from twenty to thirty-five on each side of the median stem, which branch dichotomously. The eggs (Fig. 89) are spherical, with a thin shell surrounded by the embryonic shell which is thick and radially striated,

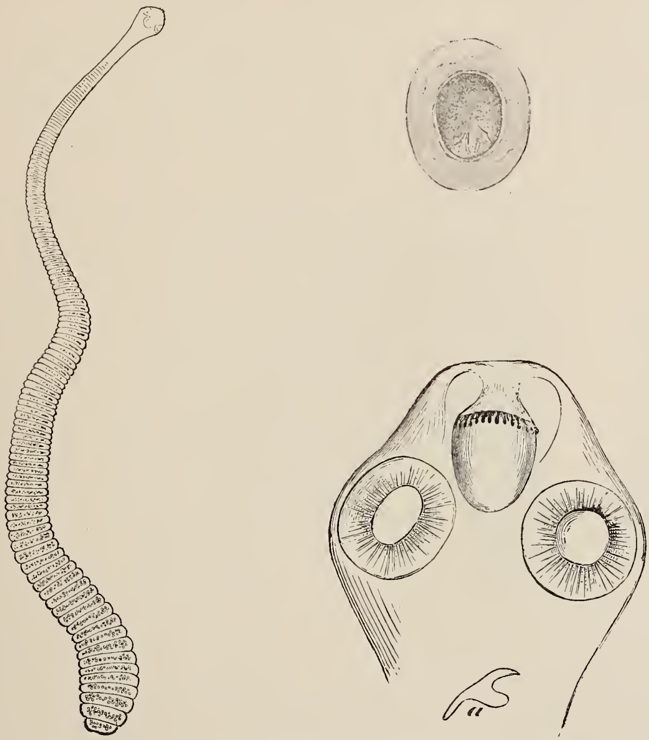


FIG. 90.—Hymenolepis nana. Adult (left), head (right), egg (above); *a*, hooklet. (From Braun.)

transparent, oval, from 30 to 40 microns long by from 20 to 30 microns wide.

HYMENOLEPIS NANA. TÆNIA NANA.—This is a dwarf tapeworm not at all uncommon in man, as Stiles[20] has shown who found it in sixteen of three thousand five hundred persons.

The worm (Fig. 90) is from 10 to 15 mm. long, from 0.5 to 0.7 mm. broad, with four suckers and a row of twenty-four to thirty very small and characteristically shaped hooks (14 to 18 mi-

[20] New York Med. Journ., 1903, vol. lxxviii. p. 877.

crons long) on the spherical head, which is 0.25 to 0.3 mm. in diameter. The segments, about one hundred and fifty in number, are short (14 to 30 microns) and relatively broad (0.4 to 0.9 mm.).

The egg is characteristic; it is spherical or oval, 30 to 37 by 48 microns in diameter, having two distinct thick membranes, each pole of the inner having a more or less conspicuous process with filamentous appendages.

The parasite inhabits the ileum, where a few or many thousands may be present. It is probably the same as the very common form in rats.

Dipylidium Caninum. Tænia Cucumerina.—This tape-worm is from 15 to 35 cm. long, from 1.5 to 3 mm. broad, the head club-shaped with rostellum and three or four rings of hooklets. The eggs are circular, from 43 to 50 microns in diameter, with thin shell. It occurs in dogs, cats, and rarely in man, and then especially children.

Bothriocephalus Latus.—This tape-worm (Fig. 91), the largest parasite of man, is exceedingly common in the maritime countries of Europe, in Ireland, and in Japan. The cysticercus stage occurs in fish. A rapidly increasing number of cases is being discovered in America. A very good report is that of Willson.[21] This tapeworm is often 8 m. in length and in some cases has reached even fifty feet. The infections are often multiple. In Willson's case there were two worms with an aggregate of eighty-two feet. In the multiple infections with fifty to one hundred worms the individuals are much shorter, sometimes from three to five feet long. The head is 1 mm. broad, from 2 to 3 mm. long, is flat, almond- or spoon-shaped, with two deep grooves at its sides which serve as suckers. It has very little neck. The ripe segments, which begin about 50 cc. from the head, increase in size until about 10 to 15 mm. broad and 3 to 4 mm. long. The genital opening is on the side, not the edge, and around it the uterus is arranged as a rosette. The distribution of these organs is more regular than that of the septa of the segments, Willson considering the imperfect or abortive segments very characteristic of this family of worms. The eggs are characteristic. They have a thin shell, the contents coarsely granular, mulberry-like, and a lid which may be open or closed. In very young eggs the lid cannot be seen, and in older may be rendered evident by pressure

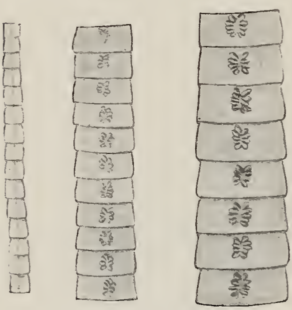


Fig. 91.—Bothriocephalus latus. (From Braun.)

[21] Amer. Jour. of Med. Sci., 1902, vol. cxxiv.

on the glass. Their size is 70 by 45 microns. The eggs are important in diagnosis, since the segments occur only at certain times, although when they do occur it is in large amounts. The most interesting thing about this enormous tape-worm is the production in certain of the hosts of an anæmia which hæmatologically cannot be distinguished from primary pernicious anæmia. Only a small percentage of cases which harbor the worm have this anæmia, and they recover rapidly when the worm is removed. Various reasons are given for the good health some hosts enjoy,—heredity, lack of predisposing causes, etc., while Dehio thinks the worm to be harmful must either die or at least be diseased. The cause of the anæmia is pretty certainly a toxine affecting the blood and perhaps the bone marrow.

The eggs of Schistosoma hæmatobium (see page 275) may also be found in the stools. It is interesting to note the number with the spine lateral and those with it terminal.

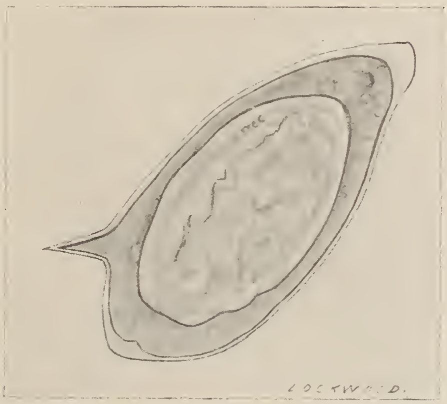


Fig. 92.—Egg of Schistosoma hæmatobium found in stool. × 400.

Plant Parasites.—Yeasts are often present in normal stools. Moulds are rare. The Oidium albicans has very rarely been found in children. Sarcinæ are often found in cases of dilated stomach, and, indeed, may lead to a diagnosis of this condition. They occur in other conditions also. When present in large numbers they may aggravate a diarrhœa by the products of their fermenting processes. They are the same as Sarcina ventriculi.

Bacteria.—The various members of the colon group—the subtilis, lactis aërogenes, and Bacillus putrificus coli—form no inconsiderable part of the mass of the stools (see page 356). Also are found Clostridium butyricum, which takes the brown stain with iodine, and with cancer of the stomach the same long bacilli as there.

Tubercle Bacilli.—In a search for these it is useless to digest a solid stool. Mucous masses should be selected, especially the blood-

stained or purulent particles, and these treated as in sputum. In class work it is interesting to note how many some find who select the particles with care, and how many students find none. In intestinal tuberculosis they are often present, and yet in cases in which they would be most expected none are found, hence the probability is that many are destroyed. Again, when found, the possibility of their origin from swallowed sputum must always be thought of, and the diagnosis of pulmonary tuberculosis has been made in this way, especially in children; but this is rather a remote possibility in the case of a careful adult.

Page's method of searching for the bacilli in the solid stool is to suspend a piece half the size of a pea in 1.5 cc. of distilled water, add 54 cc. of a mixture of equal parts of absolute alcohol and ether, and centrifugalize for ten minutes; a smear made of the sediment is fixed to the slide with a drop of egg albumin, and stained as usual.

Stools in Disease.—In *typhoid fever* the stool most characteristic is of "pea-soup" appearance, copious, watery, of foul odor, alkaline reaction, with many triple phosphate crystals. But in clinics which limit these patients to a rigid milk diet, diarrhœa is less common than constipation. The stool is frequently blood-tinged, this tingeing sometimes warning us of a coming hemorrhage. Pus (microscopic) is rare except in severe cases with extensive ulceration.

In severe *Asiatic cholera* the rice-water stools are quite characteristic. These are copious, the water being in good part secreted by the intestinal wall, with small gray flecks which are masses of epithelial cells, cholera spirilla, and fat droplets. They have no fecal odor, are alkaline, almost acholic, sometimes are blood-stained, contain little albumin and much salt.

In the mild cases there is a simple diarrhœa; in those of cholera sicca the opposite condition to the above obtains.

In *dysentery, rectal diarrhœa, and cancer of the rectum*, the movements are frequent and scanty. They soon lose their fecal character and become mucoid, mucopurulent, hemorrhagic or sero-hemorrhagic, the amount of blood separating the cases of "white" from "red" diarrhœa. Sometimes fragments of necrotic mucous membrane or of cancer are found; often masses of bloody mucus. The odor is sometimes slight, sometimes very foul.

In *amœbic dysentery* during the acute exacerbations there is diarrhœa of from three to six movements a day containing bloody mucus in which may be found the amœbæ; the stools are thin and watery, their odor offensive. These periods are separated by others of even years' duration, with normal movements, or constipation, and yet here the amœbæ may be found if looked for. It is in these cases that the

routine examination of the stool is important, especially in cases with liver troubles, as was seen in a recent case of irregular fever without liver or intestinal features and constipation. At autopsy a large amœbic abscess of the liver was found.[22]

Pancreatic Disease.—Fatty stools are common (see page 360), yet this must be confirmed by other signs of pancreatic disease. A large amount of muscle-fibre (Azotorrhœa) in a case without diarrhœa is a valuable sign; a reduction of ethereal sulphates in the urine is a point in favor, yet alone is of little value; yet all together are of value in a case without jaundice and with other signs of pancreatic disease, as abdominal tumor, glycosuria, etc. In some cases of diabetes the stool is pure fat, in amount from a few drachms to a cupful of pure yellowish-brown fat; in others about 30 per cent. is fat. Naunyn knows of no case of true fatty stools in diabetes without pancreatic disease.

For Sahli's test of the efficiency of the pancreatic juice, see page 355.

A recent method highly recommended is the use of pancreon. which preparation is not affected by the gastric juice. In a case with much muscle in the stool and no diarrhœa, if pancreon be given and the muscle diminish, it is in favor of pancreatic disease.

Some (*e.g.*, Ury and Alexander)[23] would base the diagnosis on fatty stools, many muscle-fibres in a fairly solid stool, and failure of jaundice. The suspicion is especially justified if a large amount of fluid fat separates itself from the rest of the stool. The simultaneous presence of glycosuria is rare, the absence of decomposition is not usual, nor should it be expected since there is so much albumin present, but the simultaneous steatorrhœa and azotorrhœa is important and with diabetes conclusive.

And yet azotorrhœa may fail wholly. Again, the steatorrhœa alone is not so important; with complete atrophy of the pancreas this may persistently fail; also its presence may be due to a long list of diseases affecting fat absorption as well as to a diminished amount of fat splitting, important as this is; and again in some cases with increased fat the per cent. split is normal. All will agree that stool examination is of little value unless the patient has been on a constant diet of known composition. As yet not enough work has been done to detect more than gross variations. The assimilation limit for fat in a normal case is about 350 gms. of butter; of this the loss is not over 7 to 10 per cent.; jaundice or acholia due to other diseases must not be present; the fat should not be emulsified; any diarrhœa should be checked with opium when making such alimentary tests.

[22] See also Councilman and Lafleur, Johns Hopkins Hosp. Rep., vol. ii. p. 395.
[23] Deutsch. med. Wochenschr., 1904, No. 36.

CHAPTER V

THE BLOOD

Obtaining the Blood.—A simple sharp-pointed lancet is necessary, or a needle with a cutting edge. Of the latter there are on the market several with a bayonet point. A good instrument is the ordinary Hagedorn needle, which the surgeons prefer for blood work. If nothing better is at hand, an ordinary steel pen, one nib of which is broken off, will do excellently. The one thing to remember is that the point should have a cutting edge, and not be round and sharp, not too long and slender, as is so often the case. There are on the market also holders in which separate needles can be inserted. These can be thrown away when dull, thus obviating the necessity of sharpening the instrument. Special forms have been invented, some with the needle on a spring which forces it to a certain depth, as, for instance, Francke's needle, which is quick and painless, and is to be recommended to those who cannot train themselves to give a sharp, quick blow. In another form the needle projects from a holder which serves as a guard, and hence, no matter how hard a blow is given, can penetrate only a certain distance. An illustration of this is the Daland needle. We prefer, however, a simple instrument, any needle with a cutting edge; and if the hand be at all trained, a puncture can be made deep enough to get as much blood as is necessary.

If considerable blood is desired, a hypodermic syringe should be used. A tight bandage is tied around the upper arm, and then a towel wet with warm bichloride wrapped around the elbow-joint, which has been previously cleaned up. The needle is inserted into the distended median basilic vein at the end of the elbow. For quantitative blood work, however, after the needle is inserted the bandage must be removed and circulation be allowed to return to normal before any blood is withdrawn, since the stasis has altered the concentration of the blood.

Two forms of forceps are necessary (Fig. 98), the one with the crossed points which will hold one cover-glass, and the other the ordinary straight pinch forceps, with which the second cover-glass is to be handled. In the case of the first-mentioned forceps the two arms should come in contact for the whole length of the blade, and therefore hold one edge of the cover-glass solidly. The spring of the second pair should be as weak as possible, since when large numbers of cover-glasses are to be handled a stiff pair will weary the hand considerably. Beginners prefer to handle one cover-glass with their fingers, and

although this is advised by many good authorities, we insist that it shall not be done in this clinic, because the technique is certainly better if the fingers do not touch the cover-glasses. Also after the worker has become accustomed to the forceps, he can work more rapidly and accurately with the two than if he uses his fingers for one.

The glass slides must be thoroughly cleaned for use in blood work. They have often a right and a wrong side, since they are cut from a flattened cylinder of glass; in some boxes almost all are slightly concave on one side and convex on the other, since the flattening was not perfect. If the blood specimen is on the concave side the slide will rock on the stage of the microscope, hence making it impossible to keep a field in focus, while if the specimen is put on the convex side the slide will rest firmly on its two ends.

The cover-glasses should be thin, No. 1, or preferably No. 0, and three-quarters of an inch square. Cover-glasses seven-eighths of an inch square are a little too large for blood work. In general only new cover-glasses shall be used. The reason for this is learned by bitter experience, because it is almost impossible to clean up a cover-glass so that small masses of detritus or hæmoglobin will not remain on the glass, and the student will think it pigment or some other unusual thing in his next blood specimen.

To clean this glassware considerable care is necessary. The technique will depend on the state in which they are found when bought. We have received boxes of cover-glasses and slides which it was so difficult to clean that we discarded them. As a rule, however, there is little difficulty, and to wash them off in soap and water, then clean water, then in 95 per cent. alcohol is sufficient. If necessary, they should be soaked about twenty-four hours in concentrated hydrochloric acid, then washed in water, then in 95 per cent. alcohol, then in ether. It is well to keep them either in 95 per cent. alcohol or, carefully wiped, in a glass dish. They should be handled only with forceps. For wiping them an old linen handkerchief is, we think, by far the best, since the repeated ironing has removed the most of the lint. A blood-worker should always have at hand a plentiful supply of clean glassware.

In obtaining the drop of blood considerable care should be taken to select that portion of the body which promises the best results. To always prick the ear or always one finger is a decidedly foolish habit, since in some cases the one will serve much better than any other. A general rule, however, should always be observed,—to avoid any part which is cyanosed or œdematous. We have seen counts made on the two ears at the same time in which the leucocyte count varied by 100 per cent., and the same may be said of the two hands. The ear is usually the best part to prick, since it is always within reach,

a nervous person cannot watch the worker, and it is relatively painless. In young children this last point is particularly true. And yet, if the lobe of the ear be small and thin it will be difficult to get a drop of blood from it. If the lobe of the ear is thick it is usually pricked on the flat side, it being stretched over the index-finger by the thumb and middle finger. In case, however, it is thin and considerable blood is necessary, it is well to prick on the edge and parallel to the surface. In cases of pernicious anæmia the ear usually offers much the best opportunity to get a good drop of blood. The Germans, as a rule, take the palmar surface of the ball of a finger of the left hand, and can usually get a good drop of blood. Our students, in studying their own blood, have learned to search on the anterior surface of the forearm for the pain points, and avoiding these, to always prick over a small superficial vein. In this way a good drop of blood is obtained easily and painlessly. In the case of very small children, the great toe or the heel is to be preferred. Each individual case should be studied, and before the prick is made the observer should decide what part of the body offers the best opportunities, whether the ear or the finger, the forearm or the foot, always remembering, however, that a congested or a cyanosed or an œdematous part is to be carefully avoided. It is particularly desirable, in case the patient is to be pricked at least twice a day, true of our pneumonia cases, to vary the parts selected so that they will not get sore. The patients will very soon tell us where they wish the drop of blood obtained. The needle need not be sterilized, but is first dipped into alcohol. The skin is washed off with alcohol and perfectly dried.

The method of "stabbing" varies. Some prefer a short, quick, sharp blow; others, slow pressure. Which method is to be recommended depends on the person using it. In general it may be said that in case considerable blood is desired a slow steady deep prick is better than a quick one. The patients much prefer a prick that is too hard rather than several which are not deep enough, and the unfortunate accident of going through the entire lobe is for the patient not so uncomfortable as the several light blows which beginners are apt to give. The part pricked should not be squeezed, nor should it be held in a position which will check its circulation, nor should it have been rubbed to increase circulation at that point, nor should by hard pressure the flow be aided, since by all of these methods the concentration of the blood may be slightly changed. The drops should well out. The first is wiped off, the second used. To encourage the flow a slight pressure probably does no harm, but to squeeze is not good technique. In case many drops are to be taken, the incision should be wiped occasionally with an alcohol sponge and then with a dry sponge, since this will keep the place bleeding better than a dry

sponge alone. Always ask for a history of hæmophilia, for the bleeding from even a small prick is in those cases difficult to check, and the very slightest prick will furnish enough or even too much blood.

The Examination of the Fresh Blood.—The examination of the specimen of fresh blood is very important, and should be a routine procedure in every possible case. Yet many neglect it for the study of stained specimens, which should be considered of secondary value. In the majority of cases, of course it is the stained specimens which we study, since it is not always or often convenient for the practitioner to study fresh blood. We speak now of the relative value of the two examinations, other things being equal. Sometimes information of the highest value and of a very unexpected nature is learned from a fresh preparation; more often hints are gained suggesting to the worker along what further lines to work. . He will thus make examinations of which he might not have thought, and can save himself the time of some done as a matter of routine. Some things can be studied only in the fresh specimens, and more can be better studied there than in the stained.

Technique.—The slide and cover-glass must be perfectly clean (see page 393). It is well that the slide be slightly warmed, perhaps by rubbing it rapidly with a cloth or holding it an instant near a flame, since the blood spreads much better on glass at about body temperature than it does on a cold one. The size of the drop is a matter of considerable moment. One about as large as the head of the common small black-headed pin is right in most cases. The skin is punctured, the first drop wiped off, and the second or a later picked up from the ear by means of a cover-glass held in the pinch forceps, care being taken that the cover-glass does not itself touch the skin, and is then dropped at once onto a slide. The blood should spread evenly. Under no condition should the cover-glass be pressed down or tapped with the forceps to aid spreading, since this does not make a poor specimen good, and does too great mechanical injury to the corpuscles. After the drop has once touched the slide it is needless to say that the cover-glass, even though it projects over the edge, cannot be pushed into a better position. The student should always make sure that he puts the cover-glass on the convex side of the slide, since it is exasperating to have a rocking specimen under the microscope. The drop should be so small that when well spread the blood film hardly reaches the edge of the cover. The reason for this is that the observer needs the whole of a specimen, however small, under observation; and since the distribution of cells varies at different parts of the slide, there is an advantage in a small drop.

Red Blood-Cells.—In the well-made specimen the red corpuscles will all lie singly, flat on their sides, not overlapping nor in rouleaux. But

in some cases it is important to know whether the tendency to rouleaux formation is increased or diminished, since in some diseases there is none, while in others it is so much increased that all the red blood-corpuscles are badly clumped. To test this a large drop of blood is used.

The NUMBER of the red blood-cells may be guessed at with a certain degree of accuracy by one who always uses approximately the same sized drop of blood.

The SHAPE of the red blood-cells is of considerable moment. In the circulation they may be cup-shaped, but in a well made specimen they flatten out on the glass, showing a biconcavity. When they do not flatten out well interesting pictures are gained; one looks, for instance, into the concavity of the cup the sides of which have contracted toward the centre and become almost parallel, enclosing an hour-glass-shaped orifice, the base of which is so thin that it seems absent. In the well-made specimen the cells lie perfectly flat and are quite round when alone, polygonal when in contact. Sooner or later near the edge of the specimen crenated cells are seen; that is, they become spherical and covered with small prickly points, giving the picture of a thorn-apple. These should never deceive, and yet they may in case a corpuscle presents but one of these projections, and that on its flat surface, in which case it is often mistaken for a small ring form of the malarial parasite. By *crenation*, however, is meant not alone this artefact of prickle formation, but a change in the contour of the corpuscle; that is, instead of a round disk with an even, circular margin (unless, of course, the corpuscles are crowded one against the other), the margin is uneven and shrunken, as is seen for instance in quartan and æstivo-autumnal malaria.

The presence of *poikilocytes* should at once attract attention. By these are meant corpuscles which are abnormal in size or shape of the cell as a whole (not the fine irregularities of crenation). Poikilocytes may be due (1) to technique. If the cover-glass be pressed upon, a certain number of the corpuscles will fragment into small spherical masses and small elongated rods which resemble bacilli. The ease with which this fragmentation occurs depends to a great extent on the condition of the corpuscles. If, because of disease, they are "weak," a slight injury which would not affect a normal corpuscle will cause them to break up (Stengel). In case the cover-glass has been moved after the cells have spread, they will suffer considerable distortion. (2) A specimen of fresh blood in a very warm, moist chamber, and especially if at 50° to 54° C., will present the most remarkable picture. The corpuscles lose their shape and show definite contractile movements; some will elongate considerably and move around with a vermicular motion. We have known of a

whole hospital staff studying with astonishment the gyrations of these overheated red blood-cells, sure that some new parasites had been discovered. More commonly the corpuscles under these conditions are seen to bud, and these buds to become detached and swim in the serum as microcytes. Sooner or later in such a specimen nearly all of the poikilocytes will fragment. (3) Age. When the specimen is made the corpuscles may be considered living cells, but in three or four hours death processes are visible; these appear much earlier in the blood of a diseased case. They are studied best in well-sealed specimens on a warm stage, and resemble those of the overheated specimen, except are less in degree. (4) But the poikilocytes which interest us most are cells which are misshapen when the specimen is first made, and which probably were so while in the circulation. A very few may be found in fresh normal blood. They occur in any severe anæmia, but especially in primary pernicious anæmia of even mild grade. Of the many forms, two were once supposed to be characteristic of this disease, those resembling a battledore, and the elongated or sausage forms. Poikilocytes certainly seem to have amœboid motion, at any rate, they are masses of contractile protoplasm. This is particularly seen in the small microcytes which can change their shape considerably and rapidly. (Plate I, 23-28; Fig. 93, k.)

The elasticity of the cells certainly varies. The large pale cells in anæmia look flabby; poikilocytes may be cells which were round in the circulation but with diminished elasticity, hence when the smear is made lose their shape; in lead poisoning it is said to be increased.

In the budding cells so often seen the projections may have the color of the normal cells or be paler or darker. They are attached by a longer or shorter pedicle, and often break free. Such buds which break loose and which have no hæmoglobin are supposed by many to be illustrations of the formation of some blood-platelets.

The SIZE of the red blood-cells should be noted. Normally in the adult the average diameter is 7.5 microns. The student should carefully observe the variations in size, and try to determine which small cells are preformed and which the result of his technique. The size of the cells, so uniform in the adult (and yet a few dwarfs will be found), varies much in the normal infant blood. In certain diseases also the variation is considerable; in some, as in chlorosis, there is a quite uniform diminution; in other diseases, as pernicious anæmia, the majority of the cells will be larger than normal; in secondary anæmias the size varies, large, small, and normal sized cells being present, true also of pernicious anæmia, but to a less degree; in tertian malaria it is often the size of the cell which helps us to find the parasite, a large, swollen, pale cell attracting attention to the enclosed tertian form, while a small shrunken cell may point to a

quartan or æstivo-autumnal, although in the case of the quartan the pigment and the protoplasm itself will probably first attract attention. The average size is said to be increased in jaundice, cholera, lead poisoning, and leukæmia; also in congenital heart disease and in cretinism.

Color.—The color of the corpuscles, normally of a greenish-yellow, may vary either in depth or in shade. Variations in depth of the color depend on the amount of hæmoglobin, and the trained eye will even suspect the color index from the examination of the fresh blood. Often by focussing the biconcavity is seen in normal corpuscles, but is not very evident, and in many cells cannot even be imagined. In case of a "light-weight" corpuscle, however, it is much more prominent; the corpuscles often appear to have no centre, and even a narrow ring may be all that is seen, the so-called "pessary forms." In other conditions the corpuscles seem to lack all biconcavity, and some may appear even biconvex, especially true of some microcytes.

The change in color tone is of particular interest. This seems to be due to some chemical change of the hæmoglobin. The corpuscles containing the quartan and especially the æstivo-autumnal parasites are beautiful illustrations of this, some cells which contain even a young ring-form appearing much darker than the other corpuscles and of a greenish "brassy" tone. A similar although less marked change in color is seen in microcytes and in cells fragmented by mechanical injury. The color of cells and fragments of cells seen in macrophages and phagocytes is so markedly changed, often so very green, that it is hard to believe it to be due to hæmoglobin.

In some diseases there is a quite uniform change in color. In chlorosis, for instance, nearly all of the cells are paler than normal; in pernicious anæmia a large number will seem darker than normal. In other conditions there is more variation in the shade of the corpuscle, as in secondary anæmia and in malaria. It is our custom each year to distribute to the class fresh specimens from several cases of chlorosis, primary anæmia, and secondary anæmia, and ask the students to decide from the appearance of the corpuscles from which condition the blood was obtained. They are required to make a fresh specimen of their own blood for comparison. Soon the students are able to express a pretty definite opinion concerning the nature of the case.

Nucleated reds are sometimes very easy, but often very hard, to find in the fresh specimen, the nucleus being indistinct. To any one who has studied amphibian blood this is not at all surprising, since in that blood in which every corpuscle is nucleated one is often surprised at the small number of nuclei he really sees. An occasional normoblast is seen in normal blood, but it is a pure anomaly.

The DEGENERATIONS of the red blood-cells are very important (Fig. 93). These are necrobiotic changes, which appear sooner or later according to the intravascular condition of the blood, or the treatment it receives when or after the specimen is made. The "total" degenerations have already been described. More important than these are those partial degenerations which go under a variety of names, as vacuolization, pseudo-vacuolization, pseudo-nucleation, état cribriform, globular decolorization, and more commonly Maragliano's endoglobular degeneration, or, in short, "Maraglianos." These Maragliano endoglobular degenerations are seen in normal blood usually from thirty to seventy minutes after the specimen is made. Usually they are found in the centre, but may be near the periphery of the cell. A cell may contain one or several. At this point the corpuscle appears thinner and a vacuole-like area appears which seems free from hæmoglobin (and the surrounding plasma stained). This area is usually round, although it may be elliptical, and increases toward the periphery until a mere rim of hæmoglobin-containing protoplasm may be left. Although they resemble vacuoles, they are probably areas of coagulative necrosis. These degenerated masses certainly change their shape and their position within the cell; they are extruded sometimes from the cell or remain when this goes to pieces. Whether their changes in shape are due to contractions of this degenerated protoplasm or to those of the surrounding normal protoplasm is a hard question, but the rapid motions that they make may lead the beginner to suspect that they are malarial parasites. It is not true amœboid motion, since it is not through their change of shape that they change their position. The rapidity with which these appear in the specimen, other things being equal, depends on the intravascular condition of the corpuscles. Maragliano and Castellino have used the time of the appearance of these degenerated areas as a basis to group diseases and stages of disease, giving to it a certain prognostic value. They may have laid too great stress on these degenerations, but it is certain that in almost any severe disease, and especially in the primary anæmias, they appear much earlier than in normal blood. The best description of these degenerations is that given by Maragliano and Castellino.[1] These vacuole-like areas may be mistaken for nuclei and for malarial parasites. The latter mistake, one may be sure from specimens sent to the clinical laboratories, has been responsible for several "unusual cases" of malaria which have been reported, in which it was claimed only hyalines were found. Although only the trained eye will recognize these, it may be said, in the first place, that their size differs from that of the parasite, beginning smaller and soon becoming larger; that they grow more numerous the longer one searches for them, so that the

[1] Zeitschr. f. klin. Med., 1892, vol. xxi.

student is often surprised that he should have overlooked so many "parasites" at first, since he finds them so easily later; they occur, as a rule, in the centre of the cell, although this is not at all constant; they are round or oval in shape, and, what is most important, they look more like vacuoles with very sharp edges, although not much more so than does the ring form of the hyalines; on changing the focus up or down this vacuole-like area enlarges or diminishes in size, while the parasite becomes more and less distinct; in general they are much easier to see than is the parasite; their movements may be similar to those of an amœboid organism, and their periphery may show the same wavy motion, but true amœboid motion is not present; they change position and they change shape, but they do not accomplish the former by means of the latter. Beautiful "segmenters" may be seen (see Fig. 93, *i*). In Fig. 93 the attempt was made to show these differences (contrast *a*, *f*, and *o* with *l*). In fixed specimens they show a granular structure and will take a basic stain, but have no red chromatin mass.

Maragliano considers that many so-called nucleated reds are really nothing but these degenerated cells; but the differences in size, the changes in appearance of these on changing the focus, and the distinct chromatin net-work of the nucleus should not allow this error.

Various other areas deserve particular notice; for instance the oval or ring forms, which are of the shape of a hyaline malaria ring with a circular refractive centre; these sometimes have a definite crescent shape, and are famous since once described as the parasite of measles, and more recently as that of spotted fever (see Anderson's figures). What these areas are is not clear; they change shape in a peculiar way; they do not increase in number or grow larger on standing; they occur in the greatest variety of conditions, but especially in measles (see Fig. 93, *b*).

In other cells are rod-like areas resembling bacilli, *c*. These "bacilli" may keep up a constant vibratory motion, moving practically through the whole substance of the cell, and hence the mistake sometimes made of suggesting cultures to isolate this organism is not to be wondered at. We remember one physician who was confident that these were typhoid bacilli within the cells. Others speak of them as "splits" in the cell.

Another common degeneration presents the appearance of a small cell on top of a larger and paler one, since the latter presents a dark circular area, but focussing shows them in the same plane (see Fig. 93, *e*, *h*, *g*). As a rule, this is an illustration of Ehrlich's hæmoglobinæmic degeneration, areas of condensed protoplasm, hæmoglobin separated from stroma; although superimposed cells do occur. Another example of this degeneration is in æstivo-autumnal malaria; cells are

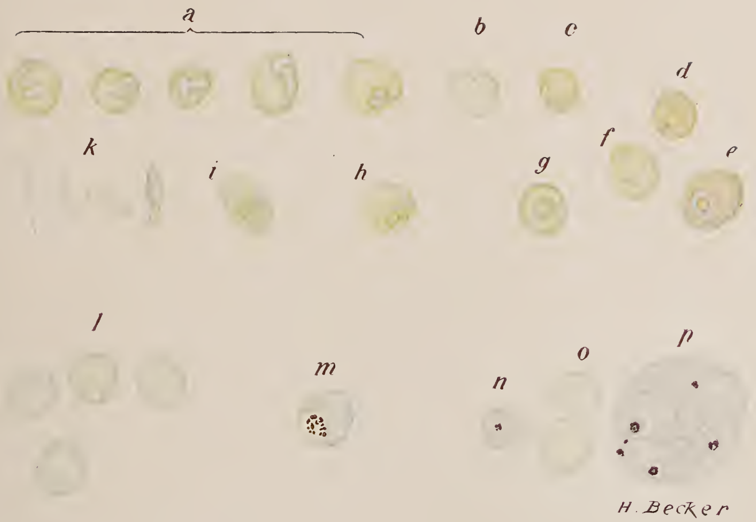


FIG. 93.—Fresh blood. *a*, cells with Maragliano's endoglobular degenerations; *b*, cell containing a navicular body, from a case of measles; *c*, the bacillus-like degeneration; *d*, a Maragliano degeneration in process of extrusion; *e*, a form of "hæmoglobin degeneration" giving a dark area; *f*, like *a*; *g*, like *e*; *h*, a degeneration like *e* but almost free from cell; *i*, a pseudo "segmenting parasite"; *k*, an "amœboid" microcyte; *l*, æstivo-autumnal hyaline malarial parasites; *m*, a full-grown æstivo-autumnal parasite, and, *n*, a segmenter, both found in the peripheral blood; *o*, same as *l*; *p*, macrophage from a case of pernicious malaria filled with malaria parasites. × 900.

seen in which the hæmoglobin seems to be gathered in a mass around the parasite (see Plate V, o). This is also seen in nucleated red cells which have the appearance of a microblast lying on a macrocyte. It is often best seen in stained specimens. Engel emphasizes this appearance in the embryo of the mouse. He thinks it indicates that the protoplasm clusters around the nucleus and separates as a small cell, leaving a large non-nucleated macrocyte, on the surface of which may sometimes be seen traces, irregularities, of the spot which the cell left. But Engel expressly states that this method of cell-formation does not occur in the latter half of pregnancy nor after birth. (The view that this nucleus with protoplasm forms a lymphocyte may be merely mentioned.) This degeneration may explain some of the "acidophilic granules" of the red cells which have been described. This degeneration is best seen in certain cases of pernicious anæmia.

The granules studied by Vaughan [2] are to be observed in the fresh blood. The skin of the finger is well cleaned with alcohol and ether, and on it is placed a drop of Unna's polychrome methylene blue. The skin is pricked through this drop, hence the blood comes in contact first with the stain. A drop of the blood thus mixed with stain is transferred to a slide and covered at once with a cover-glass. In a few minutes a few cells are seen to contain granules staining violet. These granules are coarse or fine, sometimes in a line across the cell, sometimes connected by a filament. Their occurrence is remarkably constant; in normal adult blood they are present in 0.5 to 1.8 per cent. of the red cells; and in almost exactly this percentage of cells in a great variety of diseases with little influence on the blood; in the blood of new-born, in 1 to 7 per cent. of the cells; in that of a fœtus, two and a quarter inches long, in 24 per cent.; in anæmias they are increased, in the primary pernicious occurring in even 18.8 per cent.; in general their number is parallel to that of the nucleated red cells. Vaughan gives good reasons for thinking that these are remains of the nucleus; they are not artefacts, and occur especially in normal-looking cells, in the position of the nucleus, and in conditions in which nucleated reds occur. He suggests them as a more delicate sign of anæmia than nucleated reds, but of the same significance.

Leucocytes.—The presence of a leucocytosis and its character will often be suggested by the fresh examination. The most of the surface of the specimen should be examined before forming a definite opinion of their number, since the distribution of these cells varies somewhat. Especially is this true of the so-called "stroked" specimens made by drawing the drop along the slide by another slide, a spreader, or a piece of paper.

[2] Jour. of Med. Research, 1903.

The leucocytes will be found as colorless, nucleated, amœboid or immobile cells, which do not float in the current.

Lymphocytes.—These are in size equal to, larger, or smaller than a red blood-cell; the nucleus is relatively large, round as a rule although sometimes deeply notched, and central in position; the protoplasm is scanty, sometimes hardly seen, in other cases presenting a ragged edge around the nucleus, and may appear somewhat granular; on the cold glass these cells of normal blood are not seen to move. Normally they make up from 22 to 25 per cent. of the leucocytes. On a warm stage those in lymphatic leukæmia especially are said to be amœboid.

Large Mononuclears.—These when typical are two or three times the size of a red blood-cell. The nucleus is often round, but more often oval in shape and eccentric in position. The protoplasm is very abundant and is clear. Although these cells appear non-amœboid, yet it is interesting that in malaria they are the dominant phagocytes. They are about 1 per cent. of the total number.

The "transitional cells" of Ehrlich seem to be old forms of the large mononuclears. They are the largest of all cells. The nucleus is pale and often deeply notched, giving it the so-called "saddle-bag" or the wallet-shape. The protoplasm is abundant. In some cells may be seen a few granules in the proximity of the nucleus. These constitute from 1 to 3 per cent. of the leucocytes.

Polymorphonuclear Cells.—*The finely granular cells of Max Schultze* constitute from 70 to 72 per cent. of the total number. They are from 10 to 15 microns in diameter, the size depending chiefly upon the extent to which the spherical cell is flattened out against the glass; the protoplasm is clear and filled with fine granules of a dust-like character; the nucleus has the shape either of a bent rod, a skein of fibres, or, as a rule, there seem to be several masses of chromatin matter, hence the old name polynuclear cells. These when they leave the blood-vessels are the ordinary pus-cell and are the greatest phagocytes of the body.

The *coarsely granular cells of Max Schultze* (eosinophiles) are usually a trifle smaller than the preceding. The nucleus is usually more regular, but this feature is not constant; the protoplasm is filled with coarse, blackish, very refractive granules of quite uniform size and shape, being round or slightly oval and about 1 micron in diameter. These are the most amœboid cells of the blood, and make up from 2 to 4 per cent. of the leucocyte count.

The *Mastzellen* in the fresh specimen resemble the finely granular cells. They cannot with any certainty be recognized, and yet will often be suspected. The granules are more irregular in size, some quite as large as of the coarsely granular cells, and do not fill the pro-

toplasm quite so completely; the nucleus is often trilobed. These cells are present in the normal blood to the extent of from 0.5 to 1 per cent. of the total number.

In various blood specimens the size of the leucocytes will seem to vary considerably. This depends upon the thinness of the smear, and hence the extent to which the leucocytes have flattened out. In the thick parts of the smear they will appear small, since spherical; in the thinner parts large, since flat.

Pigmented leucocytes containing blood pigment are best studied in the fresh or air-dried specimens. This pigment may be melanin, blackish or brownish granules in which no iron can be demonstrated, formed within the malarial parasite, and when set free picked up by the leucocytes. These are very important in the diagnosis of malaria. Similar granules are seen in the leucocytes in cases of melanosarcoma and then indicate a generalization of the tumor.

Hæmosiderin pigment occurs rarely in the leucocytes in cases with rapid blood destruction as ochre granules. The iron may be demonstrated by treating the smear first with 2 per cent. potassium ferrocyanide, then with 0.5 per cent. hydrochloric acid. The specimen is mounted in glycerin. The granules become blue in color.

Müller's Blood-Dust.—Blutstäubchen, or Hæmokonien granules. Müller called attention a short time ago to the presence in the normal blood of very fine granules which danced actively between the corpuscles. Finding a large number of them in a case of Addison's disease he supposed they bore some relation to that malady, but later decided that they were present in all bloods, although in very varying amount. They are described as small round colorless granules, which vary considerably in size, some one micron in diameter, but for the most part very fine and dust-like. The larger ones resemble micrococci. They are best seen by gas-light. Their nature Müller could not determine, but since they did not give the osmic acid test he said they were not fat, although they resembled it, and as they were not cleared by acetic acid he decided they were not of albuminous nature.

They were further studied by Stokes and Wegefarth,[3] who decided that they were the extruded granules of leucocytes. The reason for this opinion is that they resemble these granules in size, in man being both coarse and fine. Good additional evidence is furnished by comparative anatomy, especially the horse and rabbit, which animals have peculiar granulations in the leucocytes and similar blood-dust granules; they can be seen to escape from the leucocytes if certain reagents are added to the blood; and, lastly, the larger ones take an eosin stain. In the stained specimen the free granules are easily seen. Their relation to immunity, which point particularly interested these writers,

[3] Johns Hopkins Hosp. Bull., December, 1897.

does not concern us, but from the study of fresh and dried blood the origin they suggest seems very probable for a certain number at least of these granules. It is probably these which have been described as spores of certain parasites in the blood.

The **fat** of the blood is evident in the fresh specimens as exceedingly fine dust-like granules which would escape observation if they were not carefully looked for. These granules form a perfect cloud in the plasma in cases of lipæmia.

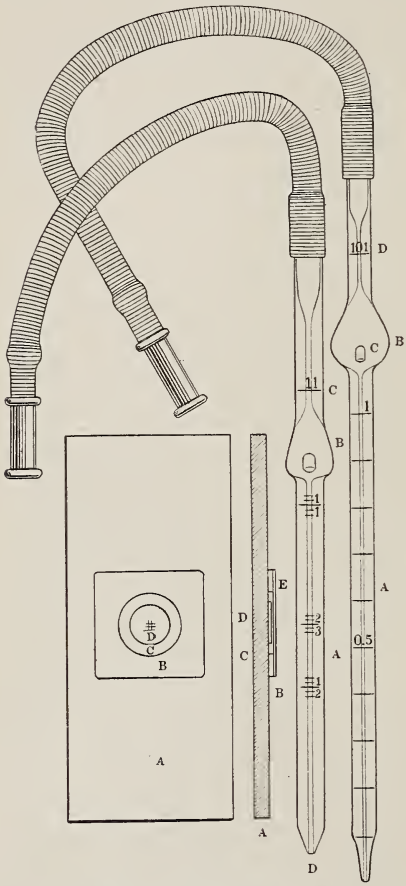


Fig. 94.—Blood-counter (Thoma-Zeiss). To the right is the ordinary form of pipette for red cells; the other is a leucocyte pipette with improved markings and point, D. The ruled counting chamber is shown on edge and face view. A, the slide; B, the ring; D, the ruled table and C, the "ditch;" E, the cover-glass.

The **platelets** are seen either singly or in large masses, or as masses of granules in the periphery of which are vacuole-like areas containing a watery fluid, the so-called "granular masses of Max Schultze." To one point we would call especial attention. In the fresh blood specimen all platelets will stick to the glass or to the corpuscles, and any floating fragment of protoplasm is certainly not a platelet, however much it may resemble it. (See page 505.)

The fresh specimens are the best in which to study the large **macrophages,** enclosing malarial parasites, red cells, and cells containing parasites in malaria, and very many red cells in typhoid fever. These cells are very poorly preserved in stained specimens. (See Fig. 93, *p.*)

In pregnancy **placental cells** (syncytium) "are commonly found" (Veit) in the mother's blood, perhaps being swept off in the blood-current.

The **fibrin net-work** should be looked for. The fibrin strands are often seen radiating from small masses of platelets. The amount is very large in certain diseases, as in pneumonia, acute articular rheumatism, *et al.*

Counting the Red Corpuscles.—The instrument to be recommended is the **Thoma-Zeiss** (see Fig. 94), which we have found uni-

formly good. In this clinic we have in constant use fifty blood-counters, and can say that the Zeiss goods never disappoint us. We have tried slightly cheaper makes, but have always been obliged to discard them. For blood-counting the best instrument is none too good.

The blood-counter consists of a PIPETTE for mixing the blood to a certain dilution, a counting chamber by means of which a layer of known depth and area is obtained, and a special cover-glass to serve as the upper boundary of this layer. The pipette is a graduated capillary tube (Fig. 94, *A*) opening into a dilatation, *B*, at the opposite pole of which empties a second shorter glass tube, *D*, to which is attached a rubber tube with a mouth-piece. This pipette is so graduated that the capacity of the reservoir to a line on the shorter tube, marked 101, is exactly one hundred times the capacity of the capillary tube from its point to a line marked 1. As a rule, the unit on the long tube is divided into ten parts, an unnecessary provision. Much to be preferred are those pipettes which have on either side of the 0.5 and the 1 marks, two smaller marks, each indicating 1/100 the length of the tube (see page 457). The end of the long tube should be obtuse, as *D*, of leucocyte pipette, since in the quick movements made it is easy to break this point. In the dilatation is a small glass ball, *C*, which aids much in mixing the blood with the diluting fluid. The pipette is to be cleaned by washing out first with water, then with alcohol, and then with ether. Air is then sucked through, not blown, until the bulb is visibly clean and on rolling the tube the glass ball rolls freely within it.

The DILUTING FLUIDS used are several in number. The one commonly used is Toisson's, the composition of which is

Water (distilled), 160 cc.;
Glycerin (neutral), 30 cc.;
Sodium sulphate, 8 gms.;
Sodium chloride, 1 gm.;
Methyl violet, 0.025 gm., or just enough to give the desired tint.

Hayem's fluid is preferred by some:

Distilled water, 200 cc.;
Sodium chloride, 1 gm.;
Sodium sulphate, 5 gms.;
Mercuric chloride, 0.5 gm.

Sodium chloride can be used in rather strong solution (3 per cent.); but it is probable that the physiological 0.6 per cent. solution will lake a certain number of corpuscles.

These fluids must always be fresh and recently filtered, since yeasts certainly do grow in those not containing an antiseptic salt, and these yeasts repeatedly lead to error.

The COUNTING CHAMBER consists of a heavy glass slide, *A*, on which is cemented a thick glass ring, *B*, the surface of which is beautifully polished. This ring surrounds a circular table of glass, *D*, the height of which is just 0.1 mm. less than that of the surrounding ring, and upon this is the ruled area. Between this glass table and the inner edge of the ring is a small ditch, *C*, to catch the drop which may run off from the table and prevent its running up between the ring and the cover-glass on the other side of the ditch. On the central glass table cross at right angles twenty-one parallel lines (see Fig. 95), equidistant, and between the extremes of which is exactly 1 mm. Hence we have an area of one square millimetre divided into four hundred small equal squares. Through each fifth row of squares is ruled an extra line. This extra line is not a boundary, but merely aids the observer to keep his position in the ruled area. Indicated, not bounded, by these extra lines, the square millimetre is divided into sixteen units of twenty-five small squares each.

In choosing a blood-counter the lines should be carefully studied, since certain makers have put on the market very imperfectly ruled slides. They should first be examined dry, to make sure that the lines are complete, and then covered with a drop of water that their sharpness may be determined; for we have seen lines which appear very distinct on a dry slide practically disappear when a drop of water covered them, since the distinctness of the line when covered with water depends not on its depth and width, but on the sharpness of the edges. If this little point is borne in mind, there will be much less dissatisfaction with blood-counters.

Before use this counting cell should be well washed with water and carefully wiped, care being taken that no lint be left on the surface of the glass ring. Alcohol and ether should never be used, since the centre glass table is cemented to the slide and is easily loosened by these reagents.

The COVER-GLASS is a heavy one with planed surface, made particularly for this use. Ordinary cover-glasses can never be used, for they are of uneven surface; they are also cut from a sheet of glass often not well flattened, and hence will not lie parallel to the surface of the glass table; and lastly, they are so thin that the capillarity of the drop will bend them down slightly.

DILUTING THE BLOOD.—After the ear, for example, has been pricked and the blood flows freely, a large drop is allowed to collect, which it should do rapidly, and is drawn into the pipette to the mark 0.5 or 1 according to the nature of the blood. For normal blood it should be drawn only to the point 0.5, in anæmic persons to the point 1. Before drawing in the blood the instrument should be tested to make sure that there is no obstruction in the tube. The blood is rapidly drawn exactly to the line desired. This will require considerable experience. If drawn too far the column may be shaken down

somewhat by tapping against a towel or rubbing it against the end of the finger, but unless there is very little correction to be made the instrument would better be cleaned up again and the whole work started anew. For this reason we prefer those pipettes with the extra marks indicating 1/100 the length of the tube, since if the column does not reach exactly the mark desired, it can be drawn to one of the other marks and then the necessary correction made. For instance, if drawn two marks beyond the 1 the worker should proceed and then diminish his final result by 2 per cent. Considerable error arises if the length of the blood column is not just right. With the long form of pipette which we use the error of 1 mm. means an error

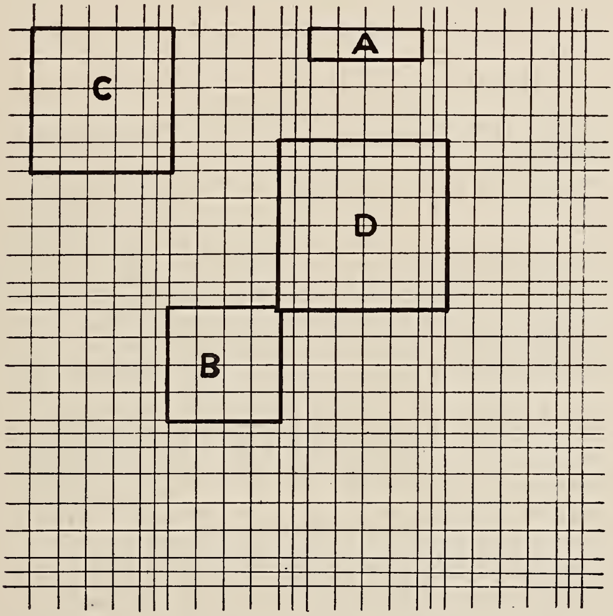


FIG. 95.—The one square millimetre ruled area, much magnified, showing the units in common use.

in our result of 2.2 per cent., and in the more common short model of pipette, one of 2.6 per cent. The mistake of 1 mm. should not be made, but it is very easy to make one of 0.5 mm., and this error of 1.3 per cent. is too great to disregard. (For these figures it is supposed that a red blood-count with the blood drawn supposedly to the 0.5 mark is being made.) After the column is at the right height the tip of the pipette is then cleaned, either on the finger or by wiping it on a towel, and the pipette plunged into a bottle of the diluting fluid. The diluting fluid is now drawn into the pipette, the tube being held vertically and rotated between the finger and thumb while the fluid enters. By this rotation the diluting fluid mixes at once with the

blood as it enters, and hence a layer of pure blood does not rise on the surface of the fluid and pass into the small tube undiluted; also in this way can best be avoided the bubble of air which often clings to the inside of the bulb. The fluid is aspirated until the mixture reaches the mark 101. It is not so necessary to accurately reach this mark, since a difference of 1 mm. in the case of the instrument now before us would mean a negligible error of only 0.03 per cent. The pipette is now withdrawn from the diluting fluid, the thumb placed over its point, and then the upper end closed by the first finger. The rubber tube may be removed or not as the worker desires. The pipette is then vigorously shaken for at least one minute. It is to be shaken in all axes except perhaps directly in the long axis of the tube, which would allow a small number of corpuscles

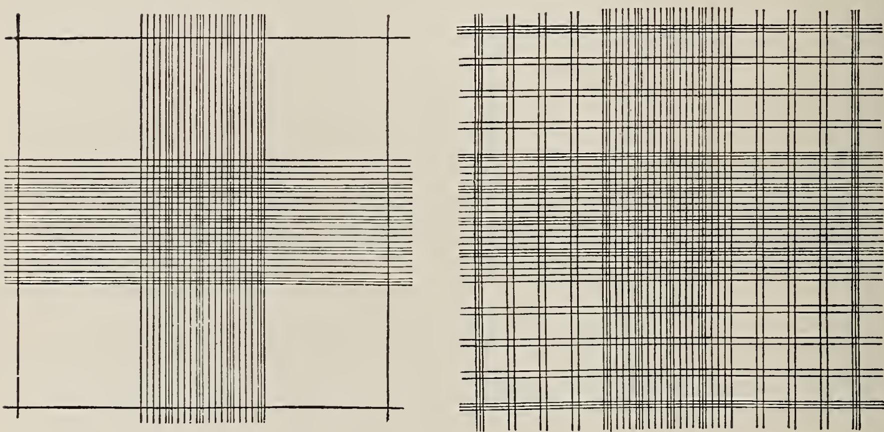


FIG. 96.—*A*, Zappert ruling; *B*, Türk's ruling.

to be shaken into the column of fluid in the capillary tube, which, of course, contains no blood. Then at once two or three drops are blown out in order to remove that column of fluid which has not entered into the mixture. It is well now to let the pipette rest for about ten minutes in order that the leucocytes may become stained by the methyl violet of the Toisson's fluid. When, however, the pipette is picked up again, the shaking should be repeated as vigorously as before. If the blood is not to be counted at once, the pipette may be sealed by stretching a rubber band over its ends. At the end of several days it may be shaken again after the fluid in the long capillary tube has been first blown out.

Filling the Cell.—After shaking again and blowing out two or three drops, a small drop, the size of which can be learned only by practice, is blown out upon the ruled table and then covered at once

by the cover-glass. The drop should not be so large as to run down into the ditch at any point, and should be large enough to almost cover the glass table. A large drop which runs into the ditch or too small a drop which merely covers the ruled surface will in either case introduce a certain error. The cover-glass should be put in position at once. The best way to do this is, we think, to grasp it by two diagonal corners, to place a third corner against the slide with the edge of the glass ring as a fulcrum, and to hold it in that position by a finger of the other hand. It is thus held up away from the drop by the edge of the glass ring as a fulcrum. By now raising the finger the cover is rotated onto the drop rapidly, and also in such a way that no air-bubble is left, which so commonly occurs if the cover be merely dropped on.

The next step is to determine whether between the cover-glass and the glass ring is any dust or dirt, which, of course, increases the thickness of the layer of diluted blood. This is done by holding the slide almost on a level with the face and toward a window, and in such a position that the light is totally reflected from the surface of the cover-glass. If the cover and slide are in good apposition, a beautiful band of colors should cover the surface of the glass ring, due to the phenomenon of interference of light so beautifully seen in superimposed layers of thin glass. Should these colors not be there the cover-glass may be touched by some instrument, but not by the point of a pencil which leaves a small amount of carbon on the glass. This may bring out the color bands, and if they remain the specimen is satisfactory. If, however, around the point of pressure appear the concentric Newton's rings, and these disappear when the pressure is removed, this slide should be cleaned up and another trial made. Certain workers seem to think that it makes but little difference if this phenomenon of light interference is not present. We consider it a very important point in the technique. When not present it usually requires considerable pressure to bring out five concentric rings, and we know that at the fifth ring the space between the cover-glass and the table is 1.4 microns, which is the thickness of one corpuscle. Over the rest of the surface, therefore, this distance must be considerable. Another method of getting the necessary contact is to allow a drop of the fluid to run under the edge of the cover, and then squeeze with the fingers the cover hard down on the slide (note the Hayem method). Dr. Cabot says that some put four small drops of the fluid, one for each corner of the cover, on the slide before the cover is put on.

This phenomenon of light interference is to many a great bugbear. It should not be so. We find it is very easy or very difficult to obtain. In the case of a well-made counting chamber it is so easy that one

attempt usually is sufficient, and from the clean appearance of the slide before the cover is put on one can predict whether or not the bands will appear. We have bought, on the other hand, counting chambers with which the bands could almost never be obtained. In handling the glass slide it should be kept as horizontal as possible, since a slight tilting may allow the cover-glass to slide off. If the cover be sealed by the fluid this will not happen. The counting chamber should now be allowed to rest for from three to five minutes that the corpuscles may settle down onto the surface of the glass, and hence render much easier the counting than if considerable focussing is necessary in order to see them all.

It will be seen that at certain points of the technique the movements must be very rapid, and it is no exaggeration to say that greater mistakes are sometimes made by too careful than by too quick work, since in trying to avoid slight errors greater ones are committed. The points of greatest importance are, the rapid filling of the pipette, and the rapid dilution with the fluid; if considerable time be spent doing this, on the slide later will be found groups of corpuscles not broken up by the shaking; the shaking must be thorough, and the drop of blood blown onto the counting chamber must be blown out at once after the shaking, and from the fluid in the interior of the bulb, not that from the capillary tube; lastly, the least possible time should elapse in putting the drop on the ruled table and covering it with the cover-glass. It is interesting to note that with the two instruments used by the French—the Hayem and the Malassey—are introduced two errors which we constantly try to avoid with the Thoma-Zeiss. Hayem recommends that an extra drop of the blood be allowed to run between the cover-glass and the table in order to seal the specimen against evaporation. Should a drop of the fluid in the case of the Thoma-Zeiss run from the ditch up between the glass ring and the slide, we would insist that the slide be cleaned up and the work done over again, yet others do not agree to this point, and think that thus is the light phenomenon more easily obtained. In the Malassey instrument is a mechanical contrivance for holding the cover-glass at a distance of 0.1 or 0.2 mm. as may be desired.

The student should learn that it takes less time to clean up his counting chamber or his pipette and begin anew than by the long counting of extra fields trying to counteract some error which he is conscious to have made. If the worker has two slides the two can be used very conveniently, the one settling while the other is being counted.

The next point is of great importance. Before counting a single cell the whole of the surface covered by the blood, even to the edge of the table far away from the ruled area, should be carefully examined

with the low power, to make sure that the distribution of cells is even. If this is not the case, no matter how even it may appear over the ruled area, the slide should be cleaned up and another preparation made.

Counting the Cells.—The power to be used in the case of the Leitz microscope is, for beginners, the 6 objective and the I. ocular. The cover-glass is usually too thick to allow of the use of a No. III. ocular. Later on the student may be able to use a 3 objective and a III. ocular. A mechanical stage is often of use, and yet it is better to train the fingers to do that work.

The unit of the ruled surface (see Fig. 95) to be used is a matter of individual preference. Cabot recommends a unit of thirty-six small squares, *D;* that is, a unit the four sides of which are rows of squares through each of which passes one of the extra lines. Simon prefers a unit of the sixteen squares, *B*, through none of which the extra line passes. Sahli recommends a unit of four squares, *A*, four of which units make up the unit recommended by Simon. We prefer a unit of twenty-five squares, *C*, on two sides of which are rows with the extra line. The reason we prefer this is that it is to this unit that the slide is ruled, and the calculation of the corpuscles is easier than with any of the others, with the exception of the Sahli, and also that there is no danger, in case we count several units on one slide, of counting any corpuscles twice, as in the case of the unit recommended by Dr. Cabot. We count usually the four corner units, of twenty-five small squares each, of one slide, and then clean up and in a new drop count the same. In this way we have counted the cells over one-half of a square millimetre. The other workers recommend a much larger number than this and all state that it were better to count four hundred small squares. In counting, cells which touch the upper and the left-hand lines are included in the unit, while cells which touch in any way the right-hand or the lower boundary lines are to be disregarded, even though all but an edge lies inside or outside the square. Since counting is usually made downward and to the right, there is less danger of counting the same cell twice. If the cell is exactly in the corner it will be necessary to remember whether that particular one has been counted once or not. The beginner should pay no attention to leucocytes, counting them as if they were red blood-cells. The reason for this is that in normal blood in eight units the probability is that but two leucocytes will be seen, an error of but 0.08 per cent., which is, of course, negligible. It is easily seen that a very high leucocytosis will introduce enough of an error to make it pay to strain the eyes to tell which are leucocytes and which are not, for although the methyl violet will stain the leucocytes fairly well, it will also stain a deep violet a certain number of red blood-cells, and for

beginners at least it is difficult to tell in many cases the nature of the cell. Hence it is better, in leukæmia for instance, to count all leucocytes with the reds, then the leucocytes with acetic acid, and the difference will be the red cells.

If the diluting fluid used be fresh or recently filtered everything seen may be counted as a cell. If spores are present they will appear like small mononuclears, and ridiculous counts due to this fact are sometimes reported. Many of the corpuscles will appear distorted, and in some anæmias very small cells are easily overlooked. They should all, however, be counted, since if the technique is good and the fluids clean only blood-cells will be seen. The high color index of pernicious anæmia has perhaps with reason been attributed to the fact that microcytes are overlooked.

The students will thus count eight unit squares each of twenty-five small squares, and the sum of these cells will be the number over ½ sq. mm. This in normal blood will be about 1250 cells. This multiplied by 2 gives the number of cells in 1 sq. mm. of a layer of blood 0.1 mm. thick; this multiplied by 10 will give the number of cells in a cubic millimetre of the diluted blood, and this multiplied by 200 (providing the blood was drawn to the 0.5 point), the number of cells in a cubic millimetre of the undiluted blood, the desired figure. In case, however, that any other units are used it takes longer to calculate the count, except in the case of the Sahli unit, which is easy since his is 1/100 of the area of the square.

The difference between the extremes of these eight figures, each the number of cells in a unit of twenty-five small squares, we do not allow in the work of beginners to be over twenty-five cells. The reason for this is as follows: With good technique the distribution of the cells will be such that the extremes of these eight figures will easily fall within that limit. If they do not, it is easier to clean up and begin over, than by counting more units, trying to offset the error of poor distribution; if they do, then it is a waste of time to count any more units. Hence by good technique at the first considerable weary counting is avoided. If one's technique is so good that he can always conform to this rule, then later he can safely report a blood-count counting only four units or even only one. He may safely do this in his private practice. In the work of the clinic, however, we do not accept such counts, although we are confident that they would be more accurate than the counts of one who has not by actual experiment learned his error and by practice corrected it, or of one who thinks it possible by counting sixteen units to offset known errors in technique.

Our rule for training the third-year men is as follows: They are to use this method until they consider themselves fairly proficient. They then count the blood of one case, usually their own, daily at the same hour on each day until the difference between two successive days is not 200,000 cells and the difference between the highest and lowest of the eight units for each day is not over twenty-five cells (a good counter will often have a difference of only thirteen or fourteen cells). Two hundred thousand cells means that we permit a difference of 4 per cent. We choose this figure not because 4 per cent. represents the error in counting, but to make due allowance for daily variations which certainly occur, and because if the two counts vary by no more than this we are sure that the error due to counting alone is less than 2 per cent. Some students attain this quickly. We have known of students, however, who must repeat this for from twenty to thirty, even sixty, times before their work was satisfactory to themselves or to us. At the end of this time they are very certain to learn wherein lies the error in their technique. It is of interest that very often it is because they are too particular and take too much time in certain steps of their work. If the reader consides that it must be an awkward man who would take thirty days to attain this accuracy, we can only say that those alone who have tested their own accuracy know how inaccurate they can be, and that some of the least successful are the most surprised to find it out. Our students are seldom guilty of reporting "rises" or "falls" of 100,000 cells, nor do they ever report a count of 4,750,600. The student who is able to conform to this rule has confidence in his technique, a confidence which is usually earned by work. He has discovered his error if any has existed, and has learned to save himself considerable eye-strain, for we know in clinical microscopy of no task more wearisome than the counting of a large number of units. Blood-counting requires considerable practice. Even the good workers after a vacation of a few weeks find that it is necessary to make trials once or twice before they are ready again for accurate work.

After the count is finished the slide should be washed with water only and the pipette rinsed first with water, then washed clean with alcohol two or three times, and then with ether twice. Air is then sucked through by mouth until the glass ball rolls easily. A suction-pump will save a great deal of trouble. The student must be careful to blow no saliva into the tube. If he draws in the alcohol before the blood is entirely removed, a precipitate will form. This may sometimes be removed by nitric acid or by filling the pipette with pepsin-hydrochloric-acid mixture and leaving it in the thermostat overnight. In case a clot obstructs the bore of the pipette, it may be dislodged with a horse-hair; we do not allow a fine wire to be used, for this will easily crack off the end of the tube.

As to the error inherent in blood-counting, we can only say the best workers have not considered it possible to count with less error than 2 per cent., and some are satisfied with 3 per cent. An error of 3 per cent. would mean that two men counting with equal accuracy the same normal blood at the same time would get results which differ by about 150,000 cells. Good workers, however, will often come much closer than this, and we know of no better way to stimulate students to attain good technique than by insisting that a certain number of them, the more the better, shall each of them with a separate instru-

ment count independently the same blood. We have seen this result in considerable extra practice.

Other Methods of Blood-Counting.—The method of **Hayem** has been used considerably, particularly in France. Hayem's fluid (see page 405) is used as dilutant.

Two cmm. of blood are measured in a small capillary tube which much resembles that of a Gowers hæmoglobinometer, and blown into a small beaker, into which has already been measured by a larger pipette 500 cmm. of the Hayem fluid. It is impossible to wash out the large pipette, and since it is found that about 6 cmm. remain in it, the blood is considered diluted 1 : 248. The blood is well mixed by means of a small glass rod. The counting cell consists of a glass chamber similar in some ways to that of the Thoma-Zeiss, but without any ruled table, the rulings being projected by a ruled chamber which fits into the substage of the microscope. The layer of diluted blood counted is a cube, each side of which is 0.2 mm., hence a volume of 0.008 cmm. He recommends that now a drop of the diluted blood be allowed to flow under the cover-glass, in order to seal the specimen. The number of cells found in this area multiplied by 248, and this by 125, will thus give the number of cells in 1 cmm. of undiluted blood.

The Malassey instrument resembles the Thoma-Zeiss in many ways. A similar mélangeur is used, and a slide with a ruled table, but the cover-glass is held by a mechanism which can be so adjusted that the layer of blood is either 0.2 or 0.1 mm. thick.

The Oliver Hæmocytometer.—This very ingenious and simple instrument is based on the principle that if blood be diluted by a fluid which preserves the corpuscles, and in a test-tube rectangular on cross-section and composed of a longitudinally striated glass, if through a suspension of opaque particles in such a glass tube a candle flame be observed, each striation in the glass will act as a lens projecting an image of the candle flame to the back wall of the tube. But this image can be formed only when the suspension is of a sufficient dilution to admit the almost unobstructed passage of the light rays. The blood is measured in a small self-filling pipette, and is washed into the tube by means of Hayem's fluid from a medicine dropper the tip of which is covered by a small rubber tube. By shaking the test-tube covered by the thumb the suspension of corpuscles is made quite uniform, but the thumb should be slid off in such a way that the drop of blood clinging to the skin will be wiped off into the interior of the tube. The tube is then held between the thumb and the first finger in such a way that these form a frame, thus eliminating extraneous rays. The tube is held close to the eye, which looks through the long axis of its cross-section at a small candle ten feet distant in a dark room. The dilution and mixing are continued until at a certain point a bright horizontal line, which is a row of images of the candle flame, is seen across the test-tube. This line appears first at the edges of the tube. The proper dilution is obtained when these two lines from the sides just meet at the centre. The tube is graduated into one hundred divisions, the 100 point being that dilution found by experiment necessary to give the end reaction in a blood of 5,000,000 corpuscles per cubic millimetre. Hence each division corresponds to 50,000 cells. The end-reaction is so sharp that students trained to it insist they can detect a variation of 12,500 cells.

It should be distinctly remembered that Oliver invented this instrument as a more accurate means of counting the blood than are the Thoma-Zeiss and similar methods. He did not invent it as a short cut for approximate blood-counting. He distinctly stated that it was not to be used in diseases accompanied by a large variation in the size of the red blood-cells. We are sure from the quite extensive use that the instrument has had in this clinic that his claim in the case of normal blood is correct, and that slight physiological variations can

be detected, which would fall far within the limits of the accuracy of the Thoma-Zeiss instrument, and the instrument is to be heartily recommended for such work. We have found, however, that in the blood diseases, as he has warned, the error is so great that the instrument cannot be used. But the clinician cares nothing about physiological variations of normal blood, and finding that it is of no use in the blood diseases, and that in the primary anæmias there may be an error of over 2,000,000 cells per cubic millimetre, he discards it as useless. We emphasize this because its use has been recommended as an approximate and easy method of counting the blood in all blood diseases, and would refer the reader to a paper by Baumgarten,[4] who emphasized the error arising from abnormal sizes of the corpuscles, and from the precipitate in the plasma.

The Hæmatocrit.—This at first promised to save considerable time and eye-strain by giving a fairly accurate determination of the volume of the red blood-cells, that is, of the hæmoglobin-containing protoplasm. The instrument is a modified centrifuge capable of very high speed. Some forms use a diluted blood; others the undiluted. In the second case in each arm of the centrifuge (see Fig. 97) is a small glass tube of rather large bore calibrated with 100 divisions. One of these glass tubes is inserted in a rubber tube with a mouthpiece and the blood drawn in until the tube is even more than full. This requires a very large drop. The finger, covered with vaseline, is then placed over the free end and then the rubber tube removed. The glass tube is inserted in the centrifuge, in the other arm of which is the empty tube to balance the machine, and at once as high a speed as possible obtained and maintained until the column of centrifugalized corpuscles does not decrease. Each division of the tube corresponds to approximately 100,000 cells. Multiplying the number of divisions by this will give an approximate blood-count and a fairly accurate estimation of the volume of the red blood-cells. This may be accurate in the case of normal blood, but as in the different anæmias in which alone blood counts are of great importance the corpuscles vary considerably not only in size, but probably in elasticity as well, it is not at all certain that they will always pack down to the same degree. At any rate, the instrument has not been very popular for blood-counting. As is so often the case, the method was used first considerably, then almost abandoned, and now is again coming into favor. Aspelin[5] centrifugalizes a blood diluted with Müller's fluid in a special pipette. The blood need not be used at once, since this mixture will keep for some time. He reads the leucocytes at the same time.

Capps[6] thinks the volume index, the numerator of which is determined with this instrument (using undiluted blood), important, and certainly has published some interesting results. We use it considerably in detecting the presence of lipæmia, cholæmia, or hæmoglobin-

[4] Johns Hopkins Hosp. Bull., July, 1902.
[5] Zeits. f. klin. Med., 1903, Bd. 49, S. 393.
[6] Jour. of Med. Research, 1903, vol. vi.

æmia. In the latter case, however, it is only safe when the plasma is free to say that hæmoglobinæmia is not present. Should the plasma be stained red one is not at all certain that this was so before the centrifugalization, for the mechanical injury to the cells may have set free a certain amount of hæmoglobin. The instrument requires very rapid work. It must be set up in close proximity to the bed. It makes a very loud and disagreeable noise, and hence is not a very satisfactory clinical instrument. To determine the volume of the red blood-cells the sedimentation by gravity in tubes to which a small amount of oxalic acid has been added to prevent coagulation is much preferred by some.

In the use of the instrument the springs holding the glass tubes in place should be occasionally tested, and the cups in which the tube rests should have at their base a piece of soft rubber; also the vaselined end should always be the distal. If these precautions be observed, the blood should remain in the tubes, but very often the considerable centrifugal force forces the whole column out of the tube.

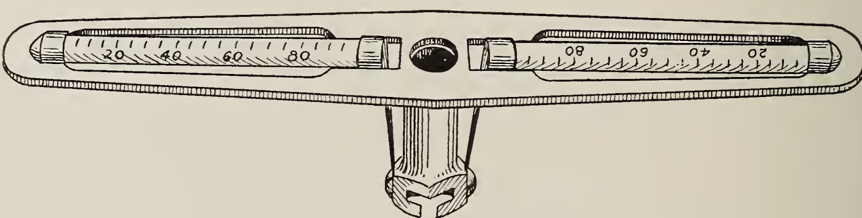


FIG. 97.—Arm of hæmatocrit.

A method which permits the specimens to be counted to be made permanent, that the enumeration may be made at leisure, is given by Strong and Seligmann.[7] The blood is diluted 1 : 100 for leucocyte counts, 1 : 20,000 for red counts, and then a quantity of known volume made into a dried and permanent mount. We judge it takes longer time than the ordinary method. Its results vary from 1 to 4 per cent. from those with the Zeiss.

Leucocyte Counting.—For a leucocyte count the same mixture in which the red cells were counted may be used, especially if Toisson's fluid was the diluent employed. In this case after counting the red blood-cells, the leucocytes over the whole square millimetre are counted. The trained eye easily picks them out, more from the difference in their refractivity than from any stain, since they are seen as bright cells when the focus is slightly raised. With the Thoma ruled slide the leucocytes in the whole millimetre field of eight separate drops should be counted. This requires about all the time at the disposal of the worker, and the number of cells counted is much too small, yet a fairly approximate result is obtained. It is much better

[7] Brit. Med. Jour., July 11, 1903.

to use a separate pipette for leucocyte counting, and a fluid which by laking the red blood-corpuscles leaves the leucocytes the only cells in the field. The best fluid is dilute acetic acid, from 0.3 to 1 per cent. A 0.3 per cent. solution is hardly strong enough, for the red blood-cells are not entirely laked, hence groups of shadows are seen which are very confusing. This does not occur if a stronger acetic acid is used. Our method is as follows: We give each student three bottles, one of glacial acetic acid, the second of distilled water, and this should be frequently renewed, and a third, a small bottle of about 30 cc. capacity with a wide neck and a label stating how many drops of the glacial acetic acid are to be added to the bottle filled with distilled water to the line of the neck to get a dilution just under 1 per cent. This mixture is made up fresh each day. A dilute acetic acid much older than this should never be used, for yeast-cells grow which if single will resemble mononuclear leucocytes. In case there are many and in chains they are at once recognized, but we know of too many instances in which a count slightly too large was reported because a few were present. The pipette used may be the same as that used in the red blood-count but the blood should be drawn to the 1 point, thus giving a dilution of 1 : 100. Better pipettes are those (see Fig. 94) which give a dilution of from 1 : 10 to 1 : 40, since the greater the number of leucocytes counted the smaller is the error. We do not agree that it is "not at all difficult" to use these big pipettes; they require more practice than the others. Their bore is so large that the blood easily drips out; it is difficult to wash the blood entirely into the bulb by means of the acetic acid; and in shaking it it is easy to shake the leucocytes into the fluid filling the tube. To reduce these errors as much as possible, while the blood is drawn and while the acetic acid is aspirated into it, the pipette should be held almost horizontal; a wide-mouthed bottle of acetic acid should therefore be used, which allows of an almost horizontal position of the pipette. The acetic acid should be sucked in rapidly, that the stream may wash the tube well. The pipette is shaken in all directions except in that of the long axis of the tube. In this case also the specimen should be first observed with the low power to make sure that the distribution is even.

If the counting slide has the Thoma ruling, hence but 1 sq. mm. for use, this area from at least eight different drops should be counted. The Ewing, Zappert, or Türk rulings are to be preferred, since 9 sq. mm. from each drop can be counted. This should be repeated with three different drops. At least one hundred leucocytes should be actually counted, and more if possible. If the acetic acid be of the proper strength and fresh, and the pipette clean, all objects seen may be counted. The number of cells found divided by the number of units

counted and multiplied by 10, and this by the dilution, will give the number of leucocytes in 1 cmm. of undiluted blood.

Beware of nucleated reds, since their nuclei are similar to small mononuclears. The hour of the count should always be stated, and also whether a short time before the count the patient had partaken of a heavy proteid meal.

The error in leucocyte counting is usually at least 5 per cent. If a large number of leucocytes are counted, as was done by Reinert, the error will be about 3.5 per cent. We are sure that the error in the ordinary blood-count made by the busy ward man is nearer 10 per cent. We wish to emphasize this fact, for too often the clinical man who does not himself count blood draws from the counts made by his assistants conclusions concerning a rise or fall of leucocytes based on differences which fall within the limits of accuracy of the method as they apply it. We hear, for instance, of a rise of leucocytes from 10,000 to 11,000 or from 20,000 to 22,000 per cubic centimetre and so on, and are confident that the blood is not nearly so much to blame for variations of this amount as was the worker. A careful man will by repeated controls of his counts make sure that his technique contains no error over 5 per cent. This can be done by filling at the same time several pipettes, which are then separately counted, or better by occasionally inviting another, in whose work he has confidence, to make a series of parallel counts with him. In control work the blood should be taken at the same time and from the same incision, for one can obtain curious results by taking his blood from different parts of the body if he does not observe due precaution to avoid the ear on which the patient has been lying or the hand which has been in a hanging position for some time.

Blood Smears.—For satisfactory stained specimens the first necessity is to get good smears, thin, with the cells well spread and only few overlapping. The method we employ is the Ehrlich, using two cover-glasses. The cover-glasses, three-quarters of an inch square and of the thinnest glass and best quality, are thoroughly cleaned in alcohol and ether (see p. 393) and then dried. One cover-glass is held on one edge by the crossed-bladed forceps. The other cover-glass is placed in a convenient position to be quickly picked up by a pair of ordinary pinch forceps. A small drop of blood about the size of a small bead (about 1.5 mm. in diameter) is picked up on the last-mentioned cover-glass which is then at once dropped onto the other cover-glass. If these covers have been properly cleaned the blood will spread out rapidly from the weight of the cover-glass alone, and without the assistance of any pressure, which should be carefully avoided. Just as the spreading of the film is about to stop, but before it does, the two covers are pulled apart in a line parallel to their plane by a steady

but quick motion (see Fig. 98). After a little practice one will succeed almost every time. Beginners find it much easier to handle the free cover-glass in their fingers. We do not allow this, since the technique is certainly not so good, the moisture from the finger affecting the specimens to a slight degree, and after one has had considerable experience one hundred or more smears, a number which must often be made for our class, can be made in much less time than without the second pair of forceps. As soon as the covers are drawn apart they may be waved in the air until dry, not warmed over a flame. They then remain spread out on a sheet of paper for from fifteen to thirty minutes to become further dried, but must be watched, for flies work havoc with such smears, sucking up the hæmoglobin and leaving large holes in the specimens. For some stains the smear is not allowed to become dry, but is dropped at once into the fixing fluid. If kept, they should be guarded from dust and moisture.

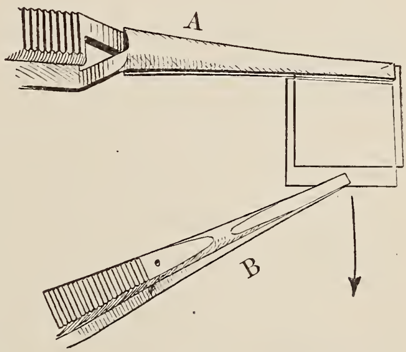


FIG. 98.—Method of making cover-glass preparations.

Others prefer to make the blood specimens on slides, in which case a cover is dispensed with. A large drop is placed on a slide and the smear made by drawing it along the slide by the edge of another slide, or, better, by a spreader with a ground edge, or, better still, by a strip of paper, the ordinary cigarette-paper giving splendid results, while others prefer to use a needle which is drawn flat across the slide. Beautiful specimens may be obtained in this way. It has the advantage of dispensing with the use of cover-glasses entirely and of giving a much larger blood surface. On the other hand only a few smears can be made; it takes more blood, and many slides cannot be handled as easily as the same number of covers. As a rule, they are not so well spread. There is great need of getting uniformly spread specimens, especially with thin edges, since the distribution of leucocytes is never uniform; these are found especially at the edges, platelets at the first point touched. A proof of this is that several smears have been sent us as illustrations of extreme leucopenia. In one case it was claimed not a single leucocyte could be found, but plenty were by those who knew the tricks such specimens play. The distribution of cells when two cover-glasses are used is not the same on each; also when the technique is slow many of the leucocytes are in a mass on the area first covered by the drop, which often gives the mistaken notion of an extreme leucocytosis.

It is of the greatest importance to obtain good specimens. With the most careful technique some of the most interesting cells are nearly always ruined. We refer to the macrophages and to the very large mononuclear cells which can be found in sections of drops of blood hardened *en masse*, and in fresh specimens.

The smears thus dried in the air may be kept some time. One obtains better results with Ehrlich's stain by heating after three or four days than on the first day, while in the case of the many methylene blue-eosin stains now in vogue it is much better to stain at once, even before they are air-dry.

Fixing Methods.—The fixing method used will depend upon the stain which it is intended to employ. Among the various chemical methods are:

(1) Nikiforoff's, consisting of absolute alcohol and ether equal parts; the specimen is to be immersed for from a half to two hours. This method is particularly good for malarial specimens and for the degenerations of the red blood-cells.

(2) Absolute alcohol for five minutes, or, if in a hurry, boiling absolute alcohol one minute.

(3) Flemming's solution, particularly good for the study of the chromatin of nuclei, consists of chromic acid, 1 per cent., 15 parts; osmic acid, 2 per cent., 4 parts; glacial acetic acid, 1 part. The blood specimens, as soon as made and before they could possibly dry in the air, are plunged immediately into this fluid and left for ten minutes. They are then washed in running water for about ten minutes and dried.

(4) Vapors of Formaldehyde.—The specimen is put under a bell-jar together with a few drops of formaldehyde, and is exposed thus to its fumes for about five minutes.

(5) The Futcher-Lazear method employs 0.25 per cent. formalin in 95 per cent. alcohol for one minute. To 10 cc. of 95 per cent. alcohol are added 4 to 5 drops of 10 per cent. formalin (*i.e.*, the strong formalin of commerce, 40 per cent., diluted with three volumes of water). This is always made fresh. The specimen is left for one minute, then rinsed in running water, and dried on filter paper.

(6) Heat.—This method, the most difficult to use well, is the only method which gives satisfactory results with the very important Ehrlich triple stain. The best apparatus is that originally introduced by Ehrlich,—a large triangular copper plate, not polished, with a gas-burner under the point. This is allowed to heat until at a constant temperature, and then the boiling point determined by dropping water at different distances from the flame until the point is reached where it just boils. The cover-glass is placed blood-side up on the copper plate inside of the boiling point, that is, toward the flame,

with its outer margin about three-quarters of an inch from the boiling point; that is, at a temperature of from 110° to 115°. Another way is to use drops of toluol or xylol instead of water; the boiling point of these is the point sought. Very good specimens may be obtained in from an hour and a half to two hours. It is better to move the specimens up to this point gradually, and at the end let them cool slowly to avoid shrinking or splitting the cells by too rapid changes of temperature. The time necessary will depend on two things: first, on the age of the specimen, for in case the blood is heated on the day that the specimen is made two hours will be hardly long enough. That it is not well to heat on the day the specimen is made is shown by the fact that the trained eye can usually tell if that has been done, by certain undesirable qualities in the specimens. In case the specimen is a week old an hour and a half is usually sufficient, and for very old specimens sometimes less than an hour. Second, on the disease which is to be studied. Normal blood requires the longest heating. Splenomyelogenous leukæmia specimens are ruined usually if left on the bar more than one hour, and a difference of fifteen minutes may be fatal to a pernicious anæmia specimen. (Our method is to place four cover-glasses at the same distance from the flame, removing the first in one hour and the others at intervals of about twenty minutes. One of these is quite certain to be good, and the rest are heated for this length of time.) Others place the blood specimen at the boiling point, but with the blood-side down, for about fifteen minutes.

A thermostat is very convenient to use, but one must be sure that the thermometer measures the temperature of the plate upon which the cover-glasses rest, rather than of the air above it. Ehrlich recommends a temperature of 110° to 120° for from an hour and a half to two hours; Engel a temperature of 125° to 135° for that same time; while Cabot, 150° for ten minutes. If in any case this high temperature be used, the glasses are placed in the oven while cool, the temperature of which is then slowly raised to 150°, the flame then removed, and the oven allowed to cool slowly.

Others prefer to simply run the cover-glass, but especially the slide, through the flame two or three times, in the same manner as smears of bacteria are fixed, and often get splendid specimens. The "Spheroidal point method" is often excellent. That point on the copper bar is determined at which the "spheroidal phenomenon" occurs; that is where the drop rolls off without boiling. The temperature here is 140° to 150° C. The smears are placed at this point face up for from thirty to one hundred and twenty seconds.

Various ovens are sold which give a very constant temperature, since they are heated by fluids whose boiling point is the one desired.

Among these may be mentioned the Viktor Meyer, and lately the Ehrlich ovens. In these ovens xylol or toluol is used.

In the case of the Ehrlich stain the success in staining will depend on the heating; if the specimen appears over-stained it was under-heated; if unstained, it was overheated. The red blood-cells are a good index; they must show no fuchsin tint whatever, nor again a lemon-yellow. The amount of stain which the plasma will take also depends on the time heated; there should be no halo around the corpuscles, under-heating bringing out Deetjen's "cell membrane."

Wood alcohol is, however, the fixative preferred by most since it can be mixed with the stain, hence the fixing requires no extra work.

In the choice of a method the subject studied must be taken into consideration. For the granulations heat is the best method, since it allows the neutrophile granules to take the specific stain; osmic acid 2 per cent. at a temperature of 37°, the specimens before they are air-dried being exposed to these vapors; or chromic acid 1 per cent., the specimen fixed for from two to ten seconds and then washed in distilled water at once for from two to ten seconds, may also be used. For the study of the nuclei, Flemming's fluid or the Nikiforoff method is best; for protoplasm, chromic acid, osmic acid, or alcohol and ether.

Stains.—Stains have been classified by Ehrlich as acid, basic, and neutral. These words do not refer to their chemical reaction, but to the portion of the compound which does the staining. The classical illustration of this is the following: ammonium picrate is an acid stain, since it is a picric acid which gives the color; rosanilin acetate is a basic stain, since it is the basic element which is efficient; as an illustration of a neutral stain rosanilin picrate would serve, since both the basic and the acid parts would stain. As a matter of fact the neutral stains are all of them mixtures of one or more stains, and it is very hard to state just how the compound arises and what it is.

Among the basic stains may be mentioned methyl green, methylene blue, fuchsin, methyl violet, Bismarck brown, and saffranin.

Among the acid, eosin, aurantia, the salts of picric acid, indulin, acid fuchsin, orange G, and a long list of others.

Neutral stains arise in mixtures of the above. For instance, of acid fuchsin and methyl green; of methylene blue and eosin.

Methods.—(1) Futcher and Lazear recommend a saturated solution of thionin in 50 per cent. alcohol, to 20 cc. of which 100 cc. of 2 per cent. carbolic acid are added. This stain is allowed to stand for some time. The specimens fixed by the alcohol-formalin method (see p. 420) are stained in this for from ten to fifteen seconds. This was particularly valuable for malaria specimens, the hyalines showing as reddish-violet ring-like bodies.

(2) Eosin and methylene blue are often convenient to use. Eosin 0.5 per cent. in 70 per cent. alcohol is diluted one-half by water, the specimens stained for a few minutes, then washed and blotted. They are then covered for three-quarters of a minute with a saturated aqueous solution of methylene blue which has also been diluted one-half just before use.

(3) Chenzinsky's stain: Methylene blue, sat. aq. sol., 40 cc.; eosin, 0.5 per cent. sol. in 70 per cent. alc., 20 cc.; distilled water, 40 cc. The specimens fixed in absolute alcohol from five to thirty minutes are stained in the thermostat at 37° C. in the above stain for from three to six hours; beautiful specimens result.

(4) Hæmatoxylin Eosin.—This stain is not used nearly as much as it deserves, since there is no better way of bringing out the nuclei. Attention has been diverted so much lately to granulations that all else has been considerably neglected.

Mayer's Solution.—Hæmatoxylin, 1 gm.; alcohol, 100 cc.; while cool, 50 gms. of alum are added, and then 1000 cc. of boiling distilled water. A few crystals of thymol are then added, the whole cooled, and filtered. It is to be kept in the dark. Only experience will tell how long the specimens are to be stained. They are afterwards washed rapidly in water. The nuclei will alone take the color. Eosin, 0.5 per cent. aqueous solution, may then be added until the red blood-cells are just rose-red. The specimen is then washed in water, dried, and mounted. The protoplasm and the nuclei are beautifully stained, but the granules not so well.

Ehrlich's mixture: Eosin (cryst.), 0.5 gm.; hæmatoxylin, 2 gms.; absolute alcohol, distilled water, glycerin, āā 100 gms.; glacial acetic acid, 10 gms.; alum, in excess. This is allowed to stand for several weeks. The specimens are stained for from one-half to two hours.

(5) Ehrlich's Triple Stain.—The words Ehrlich's "triacid" and Ehrlich's "triple" stain are often wrongly used as synonyms. The triacid stain was a mixture of indulin, nigrosin, and aurantia, equal parts of the saturated solutions. This stain was to bring out the eosinophile granules. It is hard to make up, and is now very little used. By the word "triacid" is usually meant the triple stain next to be mentioned.

Ehrlich's triple stain is a mixture of the saturated aqueous solutions of methyl green ōō, acid fuchsin, and orange G. (Grübler's stains are usually used.) These solutions are allowed to stand at least one week, and if longer give still better results.

Since only about one mixture in ten is a success, it is better to make it up in small quantities.

In a beaker are mixed: Saturated solution of orange G, 13 to 14

cc.; saturated solution of acid fuchsin, 6 to 7 cc.; distilled water and alcohol, of each, 15 cc.; saturated solution of methyl green ōō, added drop by drop, stirring all the time, 12.5 cc.; alcohol, 10 cc.; glycerin, 10 cc.

If the mixture is a success the stain has a russet-brown color, not a deep red. In case it was one of the nine failures, one may try a little doctoring with more orange G, or more methyl green, but this seldom improves matters, and those with much experience throw it away and start over.

This stain seems to improve for a time on standing, but when old certainly spoils. It should never be shaken, and should not be filtered. The drops to be used are removed on the glass rod from as near the centre of the bottle as possible. The specimen is covered with this stain for from three to twenty minutes. It is very difficult to overstain, films presenting this appearance are usually underheated. The specimen is then washed in distilled water, quickly blotted, and mounted in balsam. It may be quickly washed in absolute alcohol, which brings out the granules more clearly but makes the nuclei paler. In a successful specimen the red blood-cells will be of a buff color without the slightest shade of red; the nuclei of the leucocytes will be of a dark green, of the normoblasts almost black; the neutrophile granules will take a lilac stain, the eosinophile granules, a crimson. This is the only stain which gives a specific color to the neutrophile granules, and it is for this purpose that it was introduced. It is inferior to other stains, both for the protoplasm and for nuclei, and does not in the least stain the Mastzell granulation. While the neutrophile granulation was considered very important, this stain was uniformly used, but to get a good idea of the blood as a whole its use is limited, and other specimens should be stained by some other methods, preferably hæmatoxylin and eosin or methylene blue and eosin, or by all three, that the other points be not neglected.

Certain bloods will be found which take the stain poorly; others well. In certain diseases, particularly lymphatic leukæmia, it is almost impossible to get a good specimen with this stain, since the basic element is so markedly lacking.

There seem to be individual peculiarities in bloods. Our students are required to stain their own blood until satisfactory smears are obtained, in order that they may learn how to judge of specimens. Some students will succeed the first time; others have made even 100 to 150 specimens from their blood without getting a satisfactory stain. Other students trying these same bloods will have no better success. Certain peculiarities of the staining qualities of cells will be so marked that it is possible sometimes to recognize whose blood (of a limited number of students) is under the microscope, provided the observer has already studied that blood. During the past year, in following the work of ninety students, we were more than ever convinced as to this point. The staining qualities of bloods depend on other factors quite as much as on the fixing and staining technique

and fluids used. Why several students cannot get a well-stained specimen of one blood and will of another must depend on the bloods. We have been unable as yet to trace any relation to time of day, diet, etc.

The POLYCHROME METHYLENE BLUE-EOSIN STAINS are at present the favorites, since they are easy to use, contain the fixative, and give fairly satisfactory results. In the case of malaria they are the best stains to use; since it is only they which bring out the chromatin of the parasite. For blood smears they are satisfactory; the nuclei stain very well, also the Mastzell granulation, and the protoplasm. The eosinophile granulation can be easily recognized, and the neutrophile granules stain perhaps as well as is necessary, and may be recognized from their fine size and purplish tint. However, if one is studying granulations, he will not use this stain alone, nor, indeed, any stain containing methylene blue, which is very tricky. At least sixteen different methods [8] of making this stain have been reported, all of them modifications of the original Romanowski. The method which we use is that described by Hastings in the Johns Hopkins Hospital Bulletin, 1905, since it is one of the easiest to make up and so seldom fails.[9]

HASTINGS' STAIN.—The dry stains necessary are eosin, soluble in water, yellow (Grübler); and methylene blue (Ehrlich's rectif.) (Grübler).

Solution A = eosin 1 per cent. aqueous.

Solution B = alkaline methylene blue 1 per cent. aqueous.

Solution C = methylene blue 1 per cent. aqueous.

Solution A may be kept ready-made; solutions B and C must be made fresh.

To prepare B use warm 1 per cent. solution of dry powdered sodium carbonate. Add to it 1 per cent. of methylene blue powder and heat over a water-bath for fifteen minutes. Add 30 cc. of water for each 100 cc. of original fluid, and heat again fifteen minutes. Then pour off the solution from the residue, divide into two equal parts, and to one part add enough 12.5 per cent. acetic acid to faint acid reaction. This is best determined by placing a drop on blue litmus paper and taking as the end reaction the point at which the margin of the drop after absorption in the paper shows a faint pink. Then add the remaining unneutralized portion to this.

To mix the stain use distilled water, 1000 cc.; solution A, 100 cc.; solution B, 200 cc.; solution C, 70 to 80 cc. In adding solution C, put in 70 cc. at once, stir well, and if no precipitate is present add a cubic centimetre at a time until one appears. After the precipitate appears the stain is allowed to stand for half an hour, and then filtered through one filter. Forced filtration is usually necessary.

[8] Baumgarten, American Med., 1904, vol. vii. p. 14.

[9] See, also, Wright's Method, Jour. Med. Research, 1902, vol. ii. p. 138.

The dry residue is removed from the paper, powdered up, and may be kept in this form or dissolved in Merck's pure methyl alcohol. Seven- to nine-tenths of a gram of dry stain is usually obtained. Three-tenths of a gram dissolved in 100 cc. of alcohol gives the staining solution. In dissolving the stain it must be rubbed up with the alcohol in a mortar and pestle, as it is with difficulty soluble.

If more than nine-tenths of a gram of dry powder is obtained the resulting stain is useless. For each new lot of stain made up one must determine the relative proportions of stain and water to be used in staining and the relative lengths of time in which to let the pure and diluted stain act. Usually 2 drops of stain for one minute on the smear and then with 4 drops of water to it for four minutes gives the best result. For uniformity in the size of drops a dropper should be used. The two drops of undiluted stain for one minute fixes the specimen, which, after the addition of the water, receives its differential stain.

All of these methylene blue-eosin stains require experiment, since different mixtures by the same method require slight variations in their use, learned only by trial. The reason for accuracy of dilution is to prevent a fine black precipitate, which detracts much from the beauty of the specimen. This precipitate may be removed by slight decolorization in 95 per cent. alcohol. If too much decolorized, it is the red chromatin of the malarial parasite which suffers the first. Only the purest methyl alcohol should be used; use distilled water to wash the specimen, since tap-water sometimes ruins it.

Jenner's stain is excellent for ordinary blood work, but lacks the red chromatin-staining element. It has this disadvantage, but no advantages over the somewhat similar polychrome methylene blue-eosin mixtures, which are no harder to make up.

For basophile granules the methylene blue stains, carbol thionin, or dahlia may be used.

Another carbol thionin mixture is thionin, 0.3 gm.; absolute alcohol, 10 cc.; carbolic acid, 1 per cent., 100 cc. The fixed smear is stained two minutes, washed in water, and dried.

Ehrlich's dahlia stain consists of distilled water, 100 cc.; saturated alcoholic (absolute) dahlia solution, 50 cc.; then, on clearing, 10 to 12.5 cc. of glacial acetic acid. The specimens (heated or fixed by alcohol, etc.) are stained for from five to ten minutes.

SPECIFIC GRAVITY OF BLOOD

(1) **Gravimetric Method.** (*a*) PYCNOMETER.—This method is certainly the most accurate, but requires considerable blood (at least 5 cc. for an accurate estimation) and a very accurate chemical balance.

(*b*) Schmalz Tubes.—This method is less accurate than the above, of which it is a modification, using tubes which hold much less blood, about 0.1 cc. These tubes are about 12 cm. long and 1.5 mm. wide, slightly constricted at the ends to prevent loss of blood. A tube is well dried and weighed on a chemical balance which is accurate to at least 0.1 mg. It is then filled with distilled water and weighed, well cleaned and dried, filled with blood, and again weighed. If c equals the weight of the tube, c', the weight of the tube filled with water, c'' the weight of the tube filled with blood, then $\frac{c'' - c}{c' - c}$ = sp. gr. This method, while accurate, requires considerable skill.

(2) **Aræometrical Methods.**—In these are used fluids of different specific gravities into which a drop of blood is introduced. If the drop rises, it means that the specific gravity of the fluid is too high. If it sinks, too low. The Roy method uses a series of bottles with fluids of various specific gravities, into samples of which drops of blood are introduced until one is found in which the drop neither rises nor sinks.

In the Hammerschlag method [10] a mixture of benzol and chloroform is used, and this mixture modified until it is of the right specific gravity. A glass cylinder perfectly clean and dry (or else the drop of blood will cling to the side of the glass) is filled with a mixture of about 1058 specific gravity. The drop of blood is then introduced, best through a capillary tube bent at the end at right angles so that the drop may be blown in without giving to it an up or down motion. If the drop rises, benzol is added; if it sinks, chloroform. After each addition the fluid must be well stirred. The mixture evaporates, hence its specific gravity changes rapidly, and since there is an interchange between the blood and the fluid, it is important to work very rapidly, to confirm the result by a fresh drop of blood, and to test the specific gravity of the mixture before any evaporation has occurred. The drop of blood may be removed by filtering through linen before the specific gravity is tested. Care must be taken that no bubble of air sticks to the drop. Slight differences in temperature make considerable difference in the result. (For this reason Langlois uses that method. He changes the temperature of the fluid in which is the drop, then when the drop no longer moves, reads the temperature and reckons the specific gravity from this.)

The specific gravity of the serum may be tested by filling a tube with the blood. It is then sealed at both ends and allowed to stand upright until the serum has separated well from the clot. The tube is then broken and a drop of the serum tested in the same way as the

[10] Wien. kl. Wochenschr., 1890, p. 1018.

blood. That of the plasma may be tested by filling with blood a glass tube which has been washed out with 3 per cent. oxalic acid to prevent clotting. This is then sealed, the cells allowed to sediment, and the supernatant plasma examined. Hammerschlag considers that this small amount of oxalic acid will not affect the result.

In conclusion, the Hammerschlag method looks easy and is simple, and yet the possibilities of error from a bubble of air in a drop, the evaporation of the mixture, imperfect mixing of the two component fluids, and the change in specific gravity of the blood from contact with this mixture are great.

The specific gravity of normal blood has been variously stated. Ehrlich considers that it varies normally from 1058 to 1062, the average for man being 1059, for woman, 1056. The figures given by Piper are, for man, 1055; for woman, 1053; for children, 1051. Landois states the average is 1054, the normal limits being 1045 to 1075. Lloyd Jones places the limits at 1036 to 1068, and Hammerschlag from 1056 to 1063. It is seen from the above figures that the specific gravity of the blood of a woman is slightly less than that of a man. At birth Lloyd Jones found it 1066. It drops, reaching a minimum of 1048 to 1050 in the second year, and then rises to a maximum, which obtains between the ages of thirty-five and forty-five, of even 1058; after the menopause the average is 1054. The rise in adult life may continue to even 1066. Diet has little effect. Menstruation, Schmalz says, is followed by a slight increase. Daily variations are noted by Schmalz, the maximum between 7 and 8 A.M. being 1060.7, and from 11 A.M. to 8 P.M. 1058.8. The specific gravity for the serum and the plasma is about the same; from 1029 to 1032, an average of 1030. The specific gravity of the plasma, while much more uniform than that of the total blood, nevertheless is diminished in dropsical condition.

Using the Hammerschlag method, twenty-three of our students, normal men, ages between twenty and twenty-five, found their blood to vary from 1051 to 1065. In the case of sixteen of the twenty-three it was from 1057 to 1061; the mean of all was 1058.

In pathological conditions the specific gravity of the blood may vary from 1025 to 1068, in most cases running parallel to the hæmoglobin. It is reduced in all anæmias, especially in chlorosis. It is reduced in many cachexias, in which case the change is in the plasma, for the hæmoglobin may be practically normal. It is increased in fevers from 1057 to 1063, in cyanosis, in obstructive jaundice.

Until the introduction of the Miescher hæmoglobinometer the specific gravity was the best method for the determination of the hæmoglobin, especially in some anæmias as chlorosis, in which cases

the change in specific gravity is due almost entirely to the variations in the amount of hæmoglobin. In cases with hydræmia, however, this rule does not hold, since there the loss is also due to changes in the water of the plasma.

It has been found that 10 per cent. hæmoglobin is equivalent to 4.46 per mille specific gravity, but with the hæmoglobin the same specific gravity can vary even 13.5 per mille. If the color index is changed, the element of the stroma enters even from 4 to 5 per mille, the absolute amount of hæmoglobin being the same. In leukæmia the hæmoglobin thus estimated is too high, while in pernicious anæmia about 2 per cent. too low. In cases of hydræmia the method cannot be used at all; for instance, in cases with dropsy, anæmia from malnutrition, post-hemorrhagic anæmia, and circulatory disturbances, in which the plasma is considerably affected. In fact, about the only condition in which it has been used with good advantage is in chlorosis. The specific gravity of the plasma is fairly constant, the change in the water affecting especially the red blood-cells. This is true even in severe blood diseases, as, for instance, in pernicious anæmia. Since the Miescher instrument has come into use there is no longer very much value in this method for hæmoglobin determinations.

Dried Residue. Hygrometry.—A weighing glass with a ground-glass stopper is first carefully dried and weighed. A little blood is then introduced, the cover is put on, and it is weighed again. It is then dried for twenty-four hours, or to constant weight, at a temperature from 65° to 70° C., and then its weight determined. The solids of the blood in the case of the normal man average about 21.6 per cent.; for the woman, 19.8 per cent. The figures of Askanazy are, for man, from 20.35 to 22.89; average, 21.92 per cent.; woman, from 19.58 to 21.46; average, 20.53 per cent.

For the study of anæmias it was hoped that this would throw important light upon the condition of the blood, since it was found to run not parallel to the specific gravity nor to the count of the red blood-cells, nor to the hæmoglobin, and it thus seemed an independent element. Its value has, however, not proved as great as was hoped.

Sedimentation of the Blood.[1]—The estimation of the volume of the red blood-corpuscles would it was hoped dispense with the hard and tedious process of blood-counting, since men said, after all it is not so much the number of the red blood-cells as the volume of hæmoglobin-containing protoplasm which is important. The volume of the red blood-cells may be determined by the centrifuge method with the hæmatocrit and undiluted blood (see page 415), or the centrifuge, the blood diluted with an equal volume of potassium bichromate 2.5 per cent. or Müller's fluid. The value of the results by this method is

hardly great, since the compressibility of the red blood-cells seems to vary in different conditions.

The spontaneous sedimentation of the red blood-cells is recognized as a more accurate method than the centrifuge.

Marcano's method: Sodium sulphate solution, sp. gr. 1020, 100 cc.; sodium chloride, 1 gm.; formalin, 3 cc.

In a special pipette the blood is diluted four times with the above-mentioned fluid, and then blown into a graduated conical glass and allowed to stand twenty-four hours. The volume of the red blood-cells may then be read directly.

The normal volume is 50 per cent. In chlorosis it often runs as low as 20 per cent., while in pernicious anæmia even 9 per cent., in general depending on the count, but the determination of which it cannot replace.

Coagulation.—The results obtained have until recently varied so widely that but little confidence can be placed in them. The time required for the coagulation of the blood outside of the body depends upon many conditions, and uniformity of technique is very important; among other things it depends upon the time the blood is in contact with the tissues of the incision, the coagulation being slower from a deep cut than from a superficial one (a difference of three minutes); upon the pressure made on the skin to force the bleeding; upon the amount of blood allowed to flow; the nature of the vessel which receives it; and upon the temperature. The second drop of blood coagulates more readily than does the first, and the last drops from a wound may clot even ten minutes faster than the first, while in the same individual at the same time the blood taken from different parts of the body coagulates with different rapidities. Again, the time varies at different hours of the day; being shorter in the morning than in the afternoon; it should not be tested soon after a meal, since the time is influenced by certain foods and drugs.

Added to all these variable factors is the belief that coagulation outside of the body is in some way a different process from intravascular coagulation, so that "we cannot bring the appearance of coagulation in the living vessel into direct parallel with coagulation of blood as ordinarily understood" (Welch). For instance, in typhoid fever, anæmia, and cachexia thrombosis is common, yet the fibrin content of the blood is low; while in pneumonia and acute articular rheumatism thrombosis is seldom, and yet the fibrin content is high. Yet concerning the rapidity of clot formation in the wound, the point of greatest interest to the surgeon, the results of experiments give us the hope to obtain a fairly correct idea from our clinical methods.

The blood may be obtained from a well cleansed finger or ear. The flow must be free and pressure avoided. The first drop is wiped

off and the second used. The time is reckoned from the appearance of the drop on the skin. Only drops which well up are to be used. The hemorrhage may be retarded by pressure between drops.

Among the older methods is Hayem's, who received the blood into a graduated cylinder and considered it coagulated when the cylinder could be tilted without the blood mass changing shape. Another way was to receive a large drop on a clean side and test its consistency from time to time with a needle. Others put on a cover-glass and watched for the appearance of fibrin with the microscope. The above have been discarded, since the results were never uniform.

Vierordt's Method.—This method has simplicity to recommend it. A white horse-hair 10 cm. long is boiled in alcohol and ether. A capillary tube 5 cm. long and of 1 mm. bore is thoroughly washed and dried also in alcohol and ether. A drop of blood giving a column about 5 mm. long is received into the tube and the white horse-hair run through it. Each minute the hair is pulled slightly through the drop. The first appearance of coagulation is shown by a slight reddish stain on the hair, which after the blood is well coagulated will again appear clean. It is of greatest importance that that part of the horse-hair which is to come into contact with the blood should not have been touched with the fingers. The amount of blood should be exactly the same each time, since the coagulation time depends directly upon the amount of blood.

All results should be confirmed by a second determination.

Wright's Method.—The apparatus as commonly used consists of a dozen capillary tubes and a small vessel in which water is kept at the required temperature. The tubes are numbered and a certain amount of blood drawn into each, the time of their filling being registered. After minute intervals the tubes are examined by blowing slightly into them, and the appearance of coagulation is detected by the readiness with which the drop will move. No tube can be twice tried, hence the tubes must be examined in such an order that various intervals of time may be represented. While the tubes are waiting, they should be kept at a temperature of either 37° C. or, better still, 18.5° C. This method Ehrlich considers gives comparable results. It is one in common use, and yet Wright has more recently repudiated it, using fine capillary tubes into which a measured amount of blood is drawn and the presence of the clot detected by blowing it out onto blotting-paper.

The method now considered the best is that of Russell and Brodie, which uses the microscope. The apparatus consists of a moist chamber with a glass bottom which can be placed upon the stage of the microscope, while the upper surface is a truncated cone of glass projecting downward into the moist chamber. The lower surface of this is of a definite size (about 4 mm. in diameter), and on it is placed a drop of blood, care being taken that the drop only just covers the surface, hence is always of the same size. The glass is then quickly fitted into the moist chamber. Through the side of this chamber projects a fine tube, through which, by means of a bulb, a gentle stream of air can be directed against the blood. With the low power

of the microscope the cells are then watched as thus agitated until they are seen to move in clumps.

This method is the most accurate yet devised. The original apparatus of Russell and Brodie[11] has been modified recently, a much cheaper one devised by Pratt, in which the glass cone is dispensed with, and a still better one by Boggs. The Boggs apparatus has the advantage of a metal tube and the improved glass cone, although the peripheral jacket, in which water of a certain temperature can be circulated, is not present, nor is this very necessary. (See Fig. 99.)

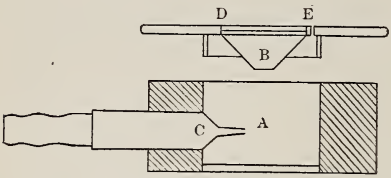


FIG. 99.—Coagulometer of Russell and Brodie as modified by Boggs. A, moist chamber; B, cone of glass the lower surface of which holds the drop of blood; C, side tube; D and E, cover-glass; at E, a pinhole.

As little blowing and at as long intervals as possible should be done. The corpuscles will at first move freely and independently of one another (see Fig. 100, *A*), then in clumps on the periphery, *B*. As the process of coagulation continues, the masses of corpuscles will no longer move in the drop, but the drop changes shape en masse, the corpuscles showing first an elastic concentric motion, *C*, and finally an elastic radial motion, *D;* that is, the current of air will cause the masses of corpuscles to move toward the

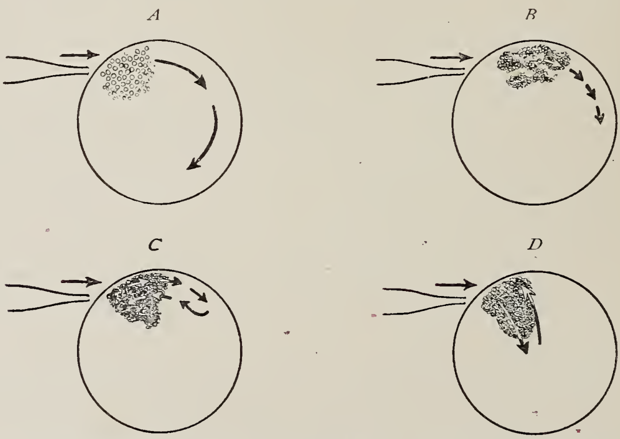


FIG. 100.—Diagram to illustrate the movement of the cells during coagulation.

centre, and to quickly spring back to their original position when the current of air ceases. This is taken as the final point, since only now can a clot be demonstrated if the disk be quickly removed and the drop be touched to a piece of filter paper. All clots should be confirmed in this way. Sometimes a "vicious circle" is set up in the drop, which clots

[11] Journ. Phys., May 12, 1897.

everywhere but one point where the blood remains fluid. Such a drop should be discarded and another attempt made. It is due to too hard blowing.

Successive records at intervals of 5 to 10 minutes should not vary over 30 to 45 seconds.

Millian's method is a modification of Hayem's. This method which is considerably in vogue among the French is to place a drop of blood on a clean glass slide, to cover it by a crystallizing dish to prevent very much evaporation, then at stated intervals to tilt the slide, and from the change in shape of the drop of blood can be determined the coagulation point. By using this method most remarkable results have been obtained, the coagulation time extending even into hours. The method has been tested under Dr. Boggs's direction in this clinic by Messrs. Hinman and Sladen, who have found that very much depends on the size of the drop of blood and the evaporation.

That coagulation time to be considered normal will depend not alone on the instrument used, but on the point considered the end. Using the Boggs instrument Messrs. Hinman and Sladen found it to vary from three to eight minutes, an average of five minutes six seconds, a longer time than some others, since they chose a later point as the end. (Brodie and Russell, three and a half minutes; Murphy and Gould, three minutes, eleven seconds; Pratt, four to five minutes.) Above nine minutes means delay in coagulation.

So many methods have been used and of such varying value that it is difficult to put in order the findings concerning the coagulation. All are, however, unanimous concerning the following point: In the hemorrhagic diatheses the coagulation time of the blood is immensely increased. In some cases of hæmophilia it requires fifty minutes, while in certain of the purpuras from ten to fifteen or more. In long-standing jaundice the coagulation time is increased, a point which interests surgeons, and in this clinic in cases of jaundice with delayed coagulation an operation is never performed until it has been decreased to about five minutes by a long course of calcium chloride. The coagulation time is diminished in stasis due to any cause, after repeated hemorrhages, transfusion, hunger, by calcium chloride, and by carbon dioxide. In this clinic the gelatin injection method for the cure of aneurism has been given a fair trial and was finally abandoned, and further work throws considerable doubt on the value of the method. In this connection it has been shown that the gelatin of commerce contains considerable calcium, and that if decalcified gelatin be used the results are quite different.

Fibrin Diagnosis.—If very thick smears of blood be made, the fibrin may be seen to radiate in strands through the specimen, usually from masses of platelets. These smears are allowed to stand for hours under the bell-jar. If desired they may then be washed by a gentle stream of water which will remove the hæmoglobin, and the fibrin

left may be stained with fuchsin and the specimen mounted. When examined fresh the specimen should be closed with vaseline to prevent evaporation. Those diseases in which most fibrin is seen are pneumonia and acute articular rheumatism. In the former case it is suggested as a differential point against tuberculous pneumonia.

BACTERIOLOGY OF THE BLOOD *

Before undertaking cultural studies on the blood the observer must have a thorough working knowledge of the principles of bacteriological technique. We shall therefore consider below only such special points as may be useful in applying general bacteriology to the study of the blood.

Technique.—The success of blood-cultures is in part dependent upon the obtaining of a sufficient quantity of blood for observation, 15 or 20 cc. being the usual amount withdrawn. In general, the median basilic or cephalic vein is chosen for the operation, the needle being passed through the skin into the vessel selected. If for any reason these be not available, a smaller vein on the dorsum of the hand or foot may be used. Incision of the skin to expose the vein, while practised by some, is not generally to be recommended, as it increases the discomfort to the patient. In very fat or œdematous individuals, however, we may be obliged to divide the skin and subcutaneous fat to find any vein large enough for use.

If the cleansing of the surface be carefully carried out, the chance of contamination by skin organisms is negligible.

Ordinarily, the site of operation is scrubbed with green soap and hot water, then rubbed over with saturated solution of potassium permanganate, followed by oxalic acid, washed with ether and alcohol, and then covered with a wet bichloride (1 in 1000) compress for an hour or more before the puncture. If there be haste and the usual materials for cleansing wanting, the skin may be briskly rubbed with a sponge of cotton or gauze slightly moistened with pure carbolic acid until a faint whitening is visible, and then plentifully washed with 95 per cent. alcohol and the culture made at once. Properly carried out, this latter method gives perfect cleansing, and usually no inconvenience to the patient, though a slight transient dermatitis with desquamation may result.

The syringe should be of the usual antitoxin type, and have a capacity of 20 cc. Care should be used in selecting one which has the glass barrel perfectly true throughout its length.

The packing of the piston should be of asbestos and very tight. Such a syringe may be boiled with impunity. In place of the ordinary washer for the needle a piece of soft black rubber tubing should be

* For this section I am indebted to Dr. Thomas R. Boggs.

cut and, after perforating with a pin, slipped over the nipple. This withstands boiling longer and gives a tighter joint.

The needle should be short and stiff, sharp, and of moderately large calibre, and may be of steel or irido-platinum. To sterilize, the syringe and needle are boiled fifteen minutes, or they may be sterilized in the autoclave. It is well to have a forceps boiled and use this in putting the needle on the syringe. Do not use the syringe until cool, as the heat may materially hasten the coagulation of the blood.

A moderately tight bandage is placed proximal to the site of operation to distend the vein, and the needle plunged through the skin, which may be anæsthetized with ethyl chloride spray, directly into the vein. The piston is drawn slowly and the syringe allowed to fill with blood. If the bandage is removed before withdrawing the needle, there will be no flow of blood to distress the patient. After withdrawal, the needle and washer are removed and the media inoculated quickly. Always pass the tip of the syringe through the flame of an alcohol lamp before inoculating each tube.

Agar tubes melted and cooled to about 45° C. are used for making plates. Bouillon and litmus milk in flasks containing 100 cc. are preferred for fluid media. Or a number of tubes may be substituted for each flask. The plates should be poured first before any coagulation has taken place.

The amount of blood in each tube or flask is varied somewhat according to the type of organisms suspected to be present, from equal parts of blood and agar to one volume of blood in five of agar; in flasks 1 to 2 cc. in 100 cc. of medium. In general, we increase the amount of blood where the feebler growing organisms as gonococcus or pneumococcus are suspected.

The members of the colon group grow better in flasks of bouillon, the pneumococcus better in milk. Anaërobic cultures may be made in the ordinary ways.

If after twenty-four hours' incubation the plates show only a few surface colonies, contamination may be reckoned upon. Only deep colonies occurring alike in several or all plates should be used for subculture. True mixed infection in the blood is uncommon. Plates and flasks should be examined daily for three or four days before discarding as sterile, as small colonies deep in the opaque media may not appear in the first twenty-four or forty-eight hours.

Value of Blood Cultures for Diagnosis.—With the increase of public and private laboratory facilities in many of our cities blood-cultures have become much more available as an aid to diagnosis. In many instances they afford the only means of accurate ante-mortem diagnosis.

The pyogenic organisms (streptococci and staphylococci) are

usually readily demonstrated in cases of general infections, osteomyelitis or malignant endocarditis being due to their presence. Some idea of the intensity of the infection may be gathered from the number of colonies per cubic centimetre of blood.

Typhoid bacilli have been demonstrated in the blood in upward of 75 per cent. of a series of cases by Cole, Buxton, Schatmüller, Hewlett, and others, often days or even weeks before the Widal test is positive.

In the paracolon infections the isolation of the organism from the blood or stools forms the only definite means of differentiation.

In pneumococcus infections the percentage of positive cultures is less but still large, the organism being found principally in the graver cases.

Among other organisms of less frequent occurrence in the blood during life may be mentioned: B. aërogenes capsulatus, B. coli, B. pyocyaneus, B. anthracis, etc.

As blood-culture involves but little inconvenience to the patient, it may be repeated if the first be negative or demand confirmation.

AGGLUTINATION PHENOMENA

Through the action of certain bacteria on the tissues there are produced in the blood soluble bodies known as agglutinins. These agglutinins, when sufficiently concentrated, have the property of clumping and rendering non-motile the specific organism whose activities gave rise to their production.

The nature of the interaction between the bacteria and the agglutinating serum is unknown. Theoretical discussion of the phenomena would carry us too far afield.[12]

Gruber-Widal Test.—This is the agglutination phenomenon applied to the diagnosis of typhoid fever.

Cultures.—A standard stock culture of B. typhosus should be kept for this purpose. An organism cultivated through many generations on artificial media is preferred.

From this stock fresh cultures on agar are grown from twelve to twenty-four hours for use in the test. Some authorities prefer fresh (ten to eighteen hours) bouillon cultures from the stock. Others use bouillon cultures killed by the addition of carbolic acid, formalin, or other toxic substances. Hastings has devised a method, based on analysis of Ficker's "Typhus diagnosticum," which yields very satisfactory, and stable-killed cultures,—viz.: To a mixture of aqueous 5 per cent. carbolic acid, 5 cc., of glycerin, 10 cc., of sterile 0.8 per cent. sodium chloride sol., 85 cc., are added the organisms scraped from

[12] For full presentation with literature, see Paltauf, Kolle u. Wassermann's Handbuch der path. Microörganismen, Bd. iv. Teil i. S. 645.

two twenty-four-hour agar slant cultures of the typhoid bacillus. The bacilli being gradually and thoroughly rubbed into the solution with a small spatula, allow to stand five or six days before using. This is used by mixing with equal volumes of the diluted sera. Living fluid cultures may give rise to confusion from the presence of clumps due to the growth of the organism. Of the dead cultures, those killed with weak carbolic are preferred, as formalin may cause precipitation of proteids from the serum in flocculi.

The emulsifying of the fresh culture from agar (rather dry slants are best) in 0.8 per cent. salt solution, or in bouillon, seems to offer the most satisfactory results. This is very readily accomplished by means of a rather stiff loop, a loopful of the growth being rubbed against the side of the tube of salt solution until thoroughly broken up and then gradually mixed with the fluid. The size of the loop gives a fairly quantitative measurement of the amount of culture used and the attainment of a suspension free from clumps is easy.

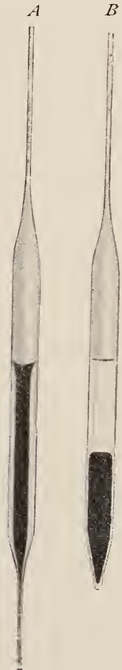


Fig. 101.—Tubes filled with clotting and clotted blood. *A*, blood is clotting spontaneously, the clot now retracting from the sides. *B*, clot in centrifugalized tube.

Collecting the Blood.—Glass tubes two inches in length by one-quarter inch in diameter are drawn out into a capillary at either end and kept on hand for the purpose. (See Fig. 101.)

From a free flowing puncture in the ear or finger-tip the blood is drawn into the tube by capillary attraction until it is two-thirds full. The tube is then placed flat on a table until the blood has clotted and the serum is separated from the coagulum. The tube is then filed and broken at a point just beyond the clot and the serum withdrawn with a capillary pipette. If a centrifuge is available, the process of separation may be hastened and the yield of serum increased by sealing the tip of tube which is free from blood in a flame and centrifugalizing a few minutes, when the clot and corpuscles will be condensed in the lower end and the serum left as a clear layer above. If it is desired to preserve the specimen or to send it away, both its ends may be sealed in the flame or with sealing-wax. Serum is best kept after separation from the corpuscles in a sterile tube. If larger amounts of blood are required, a vein should be aspirated with the syringe as in the procedure for blood-culture.

Diluting Serum.—To obtain the dilution of serum used in the reaction a number of methods are employed. A simple and very satisfactory method is as follows: A piece of one-quarter-inch glass tubing is drawn into a long capillary, as shown in cut. This is plunged into the serum in the collecting tube and the

capillary allowed to fill, care being taken to avoid stirring up the corpuscular layer. From this capillary the serum is dropped into the tubes or dishes in which the dilutions are to be made. A small water-color palette of porcelain is very convenient for making a number of dilutions. Salt-cellars or watch-crystals may be used. As a routine at least two dilutions of each serum should be made, 1 to 10 and 1 to 50.

For this purpose we proceed as follows: To the first drop of serum we add 4 drops of 0.8 per cent. salt solution dropped from *the same pipette*, which has been washed out with distilled water to remove any trace of serum, and then dried in the flame. To the second drop of serum 24 drops of salt solution are added giving dilutions of 1 in 5 and 1 in 25. Now, the addition to any portion of these dilutions of an equal volume of the suspension of the typhoid culture will give us dilutions of 1/10 and 1/50. In the same way any desired dilution may be made. If greater accuracy or very high dilutions be required, special mixing pipettes similar to the Zeiss mélangeur for blood counting may be employed. Again, dilutions may be made directly from the whole blood with such a mélangeur, using salt solution as diluting fluid and counting each two volumes

FIG. 102.—Tube used for diluting serum.

of blood as one volume of serum. The mixture is allowed to settle or, better, is centrifugalized before using. With the diluted serum we can now proceed to the macroscopic or microscopic tests.

A. MACROSCOPIC METHOD.—This method depends on the agglutination of the organisms into clumps visible to the naked eye and the eventual precipitation of the clumps, leaving a clear supernatant fluid. The serum is diluted in a test-tube of small calibre, and the organisms added either as a suspension of living or killed cultures; or, what is perhaps more convenient, the full dilution, as 1 in 50 or 1 in 100, is made with salt solution and the organisms from solid culture suspended directly in the diluted serum, as described in the foregoing section. The tube is then examined by strong transmitted light to see that its contents are homogeneous and free from accidental clumps. A narrow band of light from a lamp enclosed by a screen aids in detecting the early appearance of clumping. A positive test is reckoned if there be general clumping at a dilution of 1/50 or higher in one hour with complete precipitation, leaving a clear supernatant fluid after twenty-four hours. The reaction is hastened if the tubes are placed in the thermostat.

This method has the advantage of simplicity in that no microscope is required and that killed cultures may be employed, thus obviating

the necessity for a thermostat and culture media. The "Typhus diagnosticum" of Ficker,[13] now so widely used in Germany, is a preparation of killed cultures, the formula for which is kept secret. More complete details of this method and its results will be found in a recent paper by Borden.[14]

Several pharmaceutical laboratories in this country now make and sell killed cultures for the macroscopic Widal.

B. The Microscopic Method.—The diluted serum may be mixed with the requisite volume of the typhoid suspension by the use of pipettes, as above noted, and a drop of the mixture taken for observation on a hanging drop slide. Or we may mix the two on the cover-slip directly. To do this we use a platinum loop of stiff wire,

Fig. 103.—Widal test. Field of motile organisms. × 900.

the plane of the loop being at right angles to the handle and the diameter of the loop being constant. The loop is dipped vertically into the serum dilution and the drop so obtained placed on the centre of the cover-slip. The loop is flamed off and dipped into the typhoid suspension in the same way, and the two drops thoroughly mixed on the cover-slip. Approximately equal volumes are readily obtained by this simple method, enabling us to secure any desired dilution. The cover-slip is then inverted over the well of a hanging drop slide which has previously been ringed about with olive oil or vaseline, and the preparation is then ready for examination. The hanging drop is observed with a moderately high dry lens (Zeiss D, or Leitz 1/6 in.), and is seen best by artificial illumination. The Argand burner or oil-lamp with a yellow flame is preferred. The light is stopped down with the diaphragm so as to bring out the refractivity of the bacteria.

[13] Berl. klin. Wochenschr., 1903, No. 45.
[14] Medical News, March 18, 1905.

The freshly made hanging drop should be free from clumps and show the organisms actively swimming about in addition to their Brownian motion. (See Fig. 103.) After the lapse of one hour, if the test is positive, the organisms will be seen to be collected entirely in clumps and to have lost their motility; this at a dilution of 1/50 or higher. (See Fig. 104.) The presence of two or three free organisms in a field otherwise well clumped is considered not to vitiate the test.

It is frequently noticed that the clumping is better at the higher dilutions, while there may be very marked bacteriolysis at 1 in 10 or 1 in 20 or even higher dilutions. Many normal sera will give perfect agglutinations at 1 in 10, and show no trace of the reaction

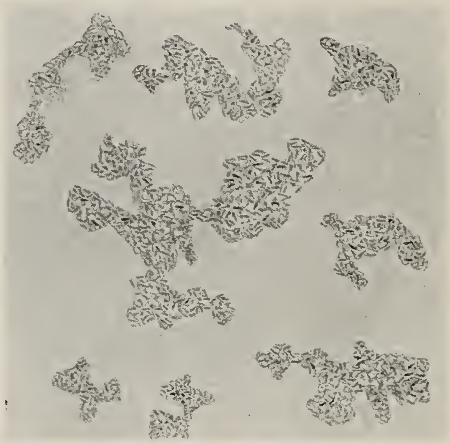


Fig. 104.—Widal test. Field of agglutinated organisms. × 900.

at 1 in 50 or higher dilutions. Hence tests based on the low dilutions alone are unreliable. The macroscopic method has rapidly gained favor in the best laboratories, and is probably less open to error than the microscopic, provided strict limits of time and dilution (one hour at dilution of 1 in 50 or higher) are observed. There is so much variation in the determination of microscopic clumping by different observers that it is often difficult to compare their results. Some authors consider any aggregation of a very few organisms agglutination. These differences have led to much confusion, particularly in experimental work.

Agglutination with Dried Blood.—This method is based on the use of blood dried on glass, tin-foil, or glazed paper, and is only accurate where the blood is carefully weighed and the dilution based on weight instead of volume. Its sole recommendation is the convenience with which the blood so dried may be transported.

Value of Agglutination Reactions in Typhoid Fever.—While the Widal reaction very rarely fails to appear in typhoid fever, it may be long delayed and is not often present before the seventh or eighth day, so that it is often no aid to early diagnosis. Still, it remains our most certain confirmatory test and is indispensable in abortive, doubtful, and obscure cases.

The persistence of the agglutinative reaction is variable, the limits being from a few weeks to many years. Some cases of long persistent Widal have been attributed to the presence of typhoid bacilli in the gall-bladder, in gall-stones, or in the urinary bladder.

The agglutination of B. typhosus by normal sera at the standard dilutions, 1/50 in one hour, is so rare as to be negligible.

The question of "associated agglutinations" in which the serum agglutinates two or more organisms closely related, as B. coli, B. alkaligines, and B. typhosus, is too complicated to find place here. Suffice it to say that the limited time and the high dilution employed in our tests is sufficient to give us reliable specific results.

Paracolon Infections.—While these often give highly specific agglutinations, the presence of associated agglutinins should be considered and the diagnosis of any one type of paracolon by agglutination reaction only would be open to error unless cultures are made for confirmation.

Other Agglutinations.—The agglutination reactions have been applied to many different organisms with more or less definite results, but in most cases they have not reached any considerable diagnostic value and are often very difficult of application.

Those specially interested will find full details in the references appended.

Dysentery group: Flexner, Bull. Johns Hopkins Hosp., 1900; also Centralbl. f. Bakt., 1901, Bd. 30. Shiga, Centralbl. f. Bakt., 1878, Bd. 23, 24.

Tubercle bacillus: Arloing and Courmont, Compt.-rend. Ac. de sc., 1898, t. 127, p. 312; Zeitschr. f. Tuberkulose, 1900, Bd. i. H. 1, 2. Fränkel, Hy. Rundschau, 1900, No. 13.

Streptococcus: Van de Velde, Arch. de Méd. exp., Paris, 1897. Neufeld, Zeitschr. f. Hygiene, 1903, Bd. 44.

Meningococcus: Jäger, Zeitschr. f. Hyg. u. Inf., 1903, Bd. 45.

Malta fever: Wright, Lancet, March 6, 1897. Strong and Musgrave, Phila. Med. Journ., 1899. In Malta fever the reaction is on a well-established practical diagnostic basis.

Paracolon: Korte, Zeitschr. f. Hyg., 1903, Bd. 44. Coleman and Buxton, Am. Jour. Med. Sci., 1902. Schottmüller, Deutsch. med. Wochenschr., 1900, p. 511.

RED BLOOD-CELLS.

The erythrocytes are specialized non-nucleated cells, which consist of hæmoglobin, 95 per cent., and stroma, and whose chief function is to carry the oxygen to the tissues, and to a lesser degree the carbon dioxide to the lungs.

In shape they are circular, discoid cells, which in well-made fresh specimens lie flat. In many of them a biconcavity is apparent, but in normal blood this must be looked for pretty sharply, and in many cells is not seen at all; in some conditions, especially the secondary anæmias, it is very evident. The opinion of Weidenreich and Lewis, that in the circulation these cells are not flat but are cap-shaped, is borne out in many specimens, especially those from the bone-marrow; clinically it is a point of no importance. Cells of normal blood, unless subjected to considerable mechanical injury in making the smear, are perfectly round and of a size varying from 6 to 9 microns in diameter. When they are not round, or are of very abnormal size, the term "poikilocyte" is applied to them (Plate I, 25–28). Such cells occur in pernicious anæmia especially, even of a mild grade, and in other anæmias of severe grade, especially in cases of cancer, tuberculosis, etc. They are probably due to alterations in the plasma.

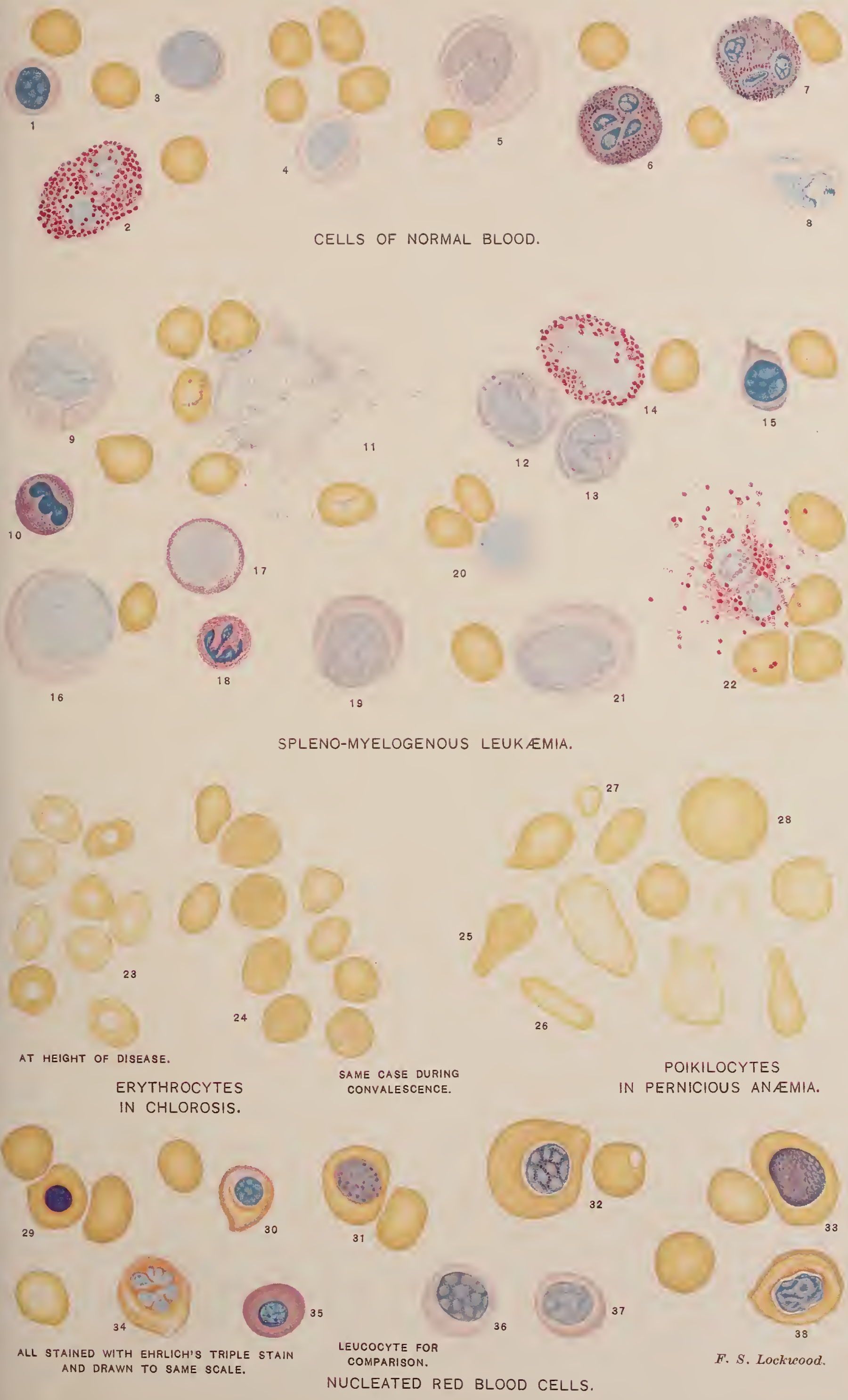
Structure.—These cells are about the hardest of all to study, being so sensitive. Various methods have been applied to demonstrate their structure, and each has shown a different one. Foà's description, a peripheral structureless hæmoglobin-containing layer, a middle with a net-work of fibres enclosing granules, and a centre of homogeneous protoplasm, is one of the most elaborate and often quoted. The consensus of opinion now is that all of the fibres, layers, etc., are artefacts; that the various granular-like bodies seen in the fresh cells are not an essential part of the cell; that those in the stained are, some at least, precipitates of the fixing agent, or of the stain; and that structure, although it certainly exists, is yet to be demonstrated. Ehrlich considers that heat is the best fixative, because it gives a homogeneous cell without structure. This argument seems weak, for heat renders difficult of observation the structure of many other cells of similar nature, including the protoplasm, but not the granules, of leucocytes, and hence may be the very worst method for the study of red cells.

Not only is their fine, but also their coarse structure in dispute. The cell membrane formerly believed to exist, then doubted, is again claimed by Deetjen, who describes it as elastic, gelatinous, glassy, and stained best in underheated specimens; while others claim merely a hæmoglobin-free concentration of the stroma at the surface.[15] Since so many believe the nucleus to disappear within the cell, they think it necessary to find some remains of it there. The question of the nucleoid is in dispute, some considering it to be related to the nucleus and others to be totally independent.[16] The word "nucleoid" has a variety of meanings in the writings of at least six observers who have employed it. It means among others the "differentiated inner body of Löwit;" that is, a nucleus-like structure in the centre of the red blood-cell which in certain specimens is very apparent, it taking a basic stain; it has a fibrillar structure, and a central clear space; in its centre again is a differentiated inner body, which "may be extruded as a platelet." "The nucleoid develops after the extrusion of the nucleus (Maximow)," but Löwit considered it the remains of the now invisible nucleus. Against these as parts of the cell is to be urged that constant technique is necessary to give constant results; that their size varies from very small to that two-thirds of the corpuscle; their periphery is indistinct often, and radially striated; that is, they do not look "genuine." It is hard to believe that Maximow can tell the age of red cells by this inner structure.

The non-nucleated red blood-cells when fresh certainly look structureless. Al-

[15] See Peskind, Am. Jour. Med. Sci., 1904, vol. cxxiv.
[16] Maximow, Arch. f. Anat. u. Physiol., 1899.

PLATE I.


CELLS OF NORMAL BLOOD.
SPLENO-MYELOGENOUS LEUKÆMIA.
AT HEIGHT OF DISEASE.
ERYTHROCYTES
IN CHLOROSIS.
SAME CASE DURING
CONVALESCENCE.
POIKILOCYTES
IN PERNICIOUS ANÆMIA.
ALL STAINED WITH EHRLICH'S TRIPLE STAIN
AND DRAWN TO SAME SCALE.
LEUCOCYTE FOR
COMPARISON.
NUCLEATED RED BLOOD CELLS.
F. S. Lockwood.

most every staining method will give a different result, and yet it is certain that they have some structure, that they have a discoplasm or the "oikoid;" a stroma which may be seen after the hæmoglobin has been washed out; but the various "inner bodies," etc., seen may be nothing but masses of degenerated protoplasm, the Maragliano endoglobular degenerations, and in the light of present knowledge all may be considered artefacts. Only perfect cells are seen in the circulation. They enter it as almost perfect, and they leave it before marked signs of age are apparent (see page 445).

Size.—In the adult these cells vary from 6 to 9 microns in diameter with an average of 7.5 microns. Hayem found that 75 per cent. varied from 6.6 to 8, 12.5 per cent. from 6 to 6.6, and 12.5 per cent. from 8 to 9 microns in diameter. These limits are quite fixed in the adult, although a very few dwarf cells do occur at all ages. But in the normal infant blood the cells vary much more, with normal limits of 3.3 to 10.3 microns. In disease the adult blood may resume this infantile condition. Evidence is given that the red blood-cells of various nationalities differ somewhat, the size diminishing as one approaches the equator. In the fresh blood occur physiological rhythmical changes, the cells being somewhat larger in the venous than in the arterial blood where they are charged with oxygen (Hamburger). Pathologically, they vary much in size.

Microcytes.—This term means cells under 6 microns in diameter. The smallest are about 3.5, and yet some are 2.2 microns. There is doubt whether all these are perfect cells or are schistocytes,—*i.e.*, fragments of larger cells,—since the process of constriction of small fragments from the red blood-cells can be followed in the fresh blood and be produced by pressure on the cover-glass, etc. For the cells above 3.5 microns in diameter we can find nucleated reds of corresponding size which represent young forms. These are seen in perfect fresh specimens. Such cells have no biconcavity as a rule, are spherical, and hence have a deep color. They occur in the embryo and infant normally, especially in premature children, and are often polychromatophilic; rarely in the healthy adult; in all anæmias, especially the primary and severe secondary.

Macrocyte is a term applied to cells from 9 to 12 microns and above in diameter; for cells from 12 to 16 microns, the term megalocyte; and for those above 16 microns, gigantocyte. These large cells are found oftenest in pernicious anæmia: some think to find them 10 per cent. of all justifies this diagnosis. They also occur in leukæmia and in chlorosis. In the last-mentioned diseases they are often of a very pale color, hence the term "chlorotic" or "dropsical" cells. They are also very common in cases with cholæmia, which is of interest since cases of pernicious anæmia are so often jaundiced (Osler). That their size is due to hydræmia is considerably in dispute; it is well known that plasma is quite constant in its water-content, and that the variations in

water of the total blood affect especially the cells. Whether the chief difference between the small dark cells and the large pale cells is the amount of water, each having approximately the same amount of hæmoglobin, is the question. In pernicious anæmia the largest cells are sometimes the darkest, and some of the microcytes are exceedingly pale, while in secondary anæmia the reverse is true. (See Plate I.)

Staining Properties.—Red blood-cells like all other cells while alive are achromatophilic, and take a stain only in proportion to their death changes. If the red cell is killed by a good fixative which prevents post-mortem changes, all normal cells are monochromatophilic, and since they take only acid stains from a mixture, are *acidophilic.* With most dyes it is the hæmoglobin especially that takes the stain, and changes in this, whether quantitative, that is, in tint, or qualitative, that is, in color tone, will show themselves to a certain degree by the tint and the tone which the cells take. Certain stains, as toluidin blue and thionin, stain the stroma especially, and hence the colors taken will vary inversely as the amount of hæmoglobin.

Cells which take other than the acid component of a stain either as a whole or in parts are termed *polychromatophilic* or *basophilic.* By this term (a synonym of which is "anæmic degeneration") we do not now include the basophilic granules to be described later. Eosin stains the basophilic cells more faintly than the normal, and if followed by a basic stain, such as hæmatoxylin, will be supplanted by it. A basic stain should not color a normal red unless it is in an old dried smear. Ehrlich's triple stain is unsuitable, since methyl green is too feeble a basic stain, and the acid components too much in excess to show the basophilia well.[17] Basophilic corpuscles are usually larger than the normal, have less biconcavity, and often are poikilocytes. With hæmatoxylin and eosin such cells take a violet tint; with the Ehrlich, a fainter tone than normal, or a grayish color; with polychrome methylene blue stains a bluish violet; in all cases, basophilic.

Ehrlich explains basophilia as a coagulative necrosis. In favor of this are that other signs of degeneration are also present; it can be produced in animals by inanition; these cells are said to be present within twenty-four hours after a hemorrhage, hence before any nucleated cells or other signs of active regeneration have begun; and it is especially the megaloblasts which are affected. The other view is that they are young cells. It is also explained as due to incomplete intracellular oxidation. Basophilic cells occur in pernicious anæmia; in the grave secondary anæmias, especially those due to cancer; in the eruptive fevers, malaria, the purpuras; and after various blood poisons.

Other cells are "*fuchsinophilic*" (Plate I, 35); that is, stained

[17] See Walker, J. of Bost. Soc. Med. Sci., November, 1899.

with Ehrlich's triple stain they are very red. Since so many of the nucleated reds of the marrow are fuchsinophilic this is considered a sign of a young cell. (These cells are usually distorted, as if very soft.) The same is said of the basophilic cells, for nearly all nucleated red cells are slightly basophilic, but basophilia and fuchsinophilia are not the same.

While the middle ground is usually unsatisfactory, yet there is, we think, the best of evidence that young red cells in general are basophilic, and good evidence that a degenerating cell will usually take such stains. The same may be said for microcytes, macrocytes, and the basophilic granules; it is hard to say whether they are signs of regeneration or degeneration, but since they occur in such a variety of conditions it is improbable that they have always the same significance. They are not signs simply of anæmia, for anæmia may be severe without them, yet in general all abnormal corpuscles—abnormal in size, shape, amount of pigment, nucleation, with protoplasm having abnormal staining affinities, etc.—are grouped as "anæmic forms," and the explanation is of little importance. To us basophilia as evidence of youth is interesting, since it was Theobald Smith who first suggested it in a case of purpura and later emphasized it in his studies on Texas fever.[18] Walker found such cells in the normal blood of all lower vertebrates; and in the fœtus of the dog and guinea-pig even ninety times as many as in the blood of the mother. In normal marrow the basophilia is in inverse proportion to the amount of hæmoglobin that the cells contain, judging by the fresh specimen before staining, hence the term "anæmic degeneration;" but this lack of hæmoglobin could be primary or result from the loss of some from the cell.[19] Walker suggests that these "cells hurried into the circulation while too young" are as good an index of anæmia as the blood-count, and their detection much easier. Germani also emphasizes them as an important feature of the blood picture in severe anæmias, their number being in direct proportion to the severity of the case, and, since easy to stain, suggests them as a valuable hint in diagnosis and prognosis.

Engel's opinion that the fuchsinophilic cells represent a related yet different type from orthochromatic cells (*i.e.*, cells staining in the usual manner), and that they send cells into the blood-stream only in anæmia when the supply from the orthochromatic nucleated reds is exhausted, has not received much support. Taylor says fuchsinophilic cells are not found in embryonic or infantile blood. We would object to this most emphatically, since that is where we have found the best illustrations of these cells.

[18] Walker, loc. cit.

[19] See also Stengel, Contrib. from Pepper Lab., Univ. of Pa., 1900.

Partial polychromatophilia is best illustrated by the Maragliano endoglobular degeneration (see p. 399), these degenerated areas staining well with a strong basic dye, especially methylene blue. The probability is that many of the so-called "inner bodies," "nucleoids," and other so-called evidences of cell-structure are nothing but these stained areas of degenerating protoplasm, which sometimes resemble malarial parasites, and the extrusion of which gives rise to bodies resembling platelets. Their resemblance to malarial parasites is so striking that we know one eminent pathologist who admitted that had he not been sure that before death a patient had not malaria he would have been unable to say that the inclusions in certain red blood-cells stained with hæmatoxylin and eosin were not malarial parasites. These degenerations certainly led to many mistaken diagnoses before the chromatin-staining mixtures were used.

The ring bodies described in some of the red cells of anæmic blood by Cabot,[20] and which require for their demonstration the polychrome methylene blue-eosin mixtures, are, he suggests, nuclear remains. They are red rings, ovals, or bands, evidently not related to the stippling of the cells. They occur especially in pernicious anæmia, but also in the leukæmias and various secondary anæmias.

In heated specimens, if this be done too quickly, or if they be exposed to moisture, around the periphery of the cell may sometimes be seen a row of large dots, which are neither true granules nor do they resemble the granules in malaria.

In certain cases of malaria (those we have seen have all been tertian and from the Tropics) the infected cells show a remarkable granulation (Plate III, 10, 13). They contain granules which are of quite uniform size, which are as coarse as the eosinophile granules, and stain purple in the Hastings' stain, while the rest of the cell stains paler, in fact may be almost colorless, as if the hæmoglobin had been condensed into these dots. (This is merely a "descriptive explanation.") We have seen cells in which the granules appeared hung in a hyaline envelope around the parasite. They can be seen in the fresh unstained cell; the lead granules cannot (Boggs). We are sure these are not artefacts, and that they are not the same as the granules of lead poisoning which also may be present in the cells. One who has seen both will not identify them.

The "methylene blue degeneration of Ehrlich" is the name given to a beautiful picture seen in specimens of fresh blood stained by this dye, the cell containing a mesh-work of fibres.

"VITAL BLOOD-STAINING."—To study these granules and fibres in the unfixed cells, one puts on the wet smear a granule of methylene blue or neutral red, then the cover is sealed at once to the slide with

[20] Journ. of Med. Research, 1903, vol. iv. p. 15.

paraffin, and the beautiful threads of fine granules are soon seen. Whether these are preformed, or signs of death or degeneration, or merely precipitates of stain in the cell, is uncertain.

Another excellent method of vital blood-staining was used by Rosin.[21] A cover-glass is lightly spread with the saturated alcoholic solution of methylazur or of toluidin blue, which is then allowed to dry. Over this stained surface a blood smear is made, and the surface at once inverted over a hollow slide with vaselined rim. The blood can be watched for even twenty-four hours. These methods are not used nearly enough.

Various poisons, potassium chlorate, pyrogallic acid, *ct al.,* often will produce vacuole-like areas or clumps in the cells, which are motile and which may break free from the cell, or the cell may be dissolved leaving them free. Heinz and Bloch describe these as "areas of poisoned protoplasm."

The Basophilic Granulation of Grawitz (Plate II, 22, 24, 25).—In certain conditions, especially lead poisoning, pernicious anæmia, leukæmia, etc., certain of the red blood-cells when stained with any good basic stain, particularly gentian violet or methylene blue, contain minute granules. They are not seen in fresh unstained specimens; they are not increased by allowing the blood to stand.

While any methylene blue-eosin mixture will do, the most beautiful specimens are prepared as follows. The air-dried smears are fixed for from three to five minutes in absolute alcohol, washed in water, and while still wet are stained with Löffler's methylene blue for a few seconds or much longer, then dried, or examined in water. The bluish-black granules stand out against the clear green corpuscles.

A beautiful stain to differentiate these from fragments of the nucleus, which many suppose them to be, is that of Pappenheim (Boellke).

Stain I. Acid. carbol. liquefact., 0.25; aqua dest., 100; methylene green (pur.), 1.

Stain II. Acid. carbol. liquefact., 0.25; aqua dest., 100; pyronin (pur.), 1.

Fifteen cc. of I. and 35 cc. of II. are well mixed and filtered. The blood-smear fixed by heat (not alcohol) is stained for a few seconds with the filtrate. The fragments of nuclei are deep greenish-blue, the granules bright red.

In a severe case one finds even five or six of these "stippled cells" in a field, but, as a rule, one must search several fields for one. There may be only one or a few granules in a cell, but as a rule the cell is well sprinkled, and even to such a degree that some consider the uniform tone of a polychromatophilic cell is due to their abundance. They may be from dust-like size to granules a micron or more in diameter. They occur anywhere in the cell, but are distributed quite regularly as a rule, perhaps more at the edge, most think in the external layers of the protoplasm. They occur in the severest anæmias, especially the primary pernicious, in which they are large and conspicuous; in sec-

[21] Rosin and Bibergeil, Zeitschr. f. klin. Med., 1904, vol. liv. p. 197.

ondary anæmia due to cancer, especially of the gastro-intestinal tract; in cachexia; in leukæmia, in which cases they are not numerous; and in septic processes; while in chlorosis some find them rare, others (Stengel and Pepper, in 11 of 18 cases) common. They also occur in phthisis, lues, chronic parenchymatous nephritis, small contracted kidney, cirrhosis of the liver (Grawitz). In gout they are many in number, and yet in rheumatism with even severe blood changes they are very rare. Few are found in tuberculosis, typhoid fever, pneumonia, lues, nephritis, etc. In gout it is of interest that especially large numbers are found in those cases with hæmatoporphyrinuria; Guyot found them regularly in the hæmoglobinuria due to cold; in tuberculosis Grawitz says they occur only after the secondary infection of a cavity. That condition in which they occur in the largest numbers is lead poisoning. They are found in the blood of Europeans who have recently moved to the Tropics.

Grawitz interpreted them as areas of coagulated necrosis and they commonly now bear the name "Grawitz basophilic granular degeneration."[22] White and Pepper,[23] Stengel and Pepper,[24] Bloch, and others agree.

On the other hand they are normal in embryonic blood, never, some say, in adult blood; nucleated reds often contain them, good evidence against their relation to a degenerating nucleus. They are often present in the degenerated reds, but also occur independently of other degenerations, as polychromatophilia, poikilocytosis, etc. Their relation to malaria and to polychromatophilic degeneration is now generally abandoned. Others say that they are in some way or other related to new formation of cells, while now and again recurs the view that they are related to the nucleus.

Cadwalader distinguishes three groups of granulated corpuscles; those with the granules in fine and coarse thread-like strands; those with fine dot-like granulations; and those with dense coarse masses. The first type is found in small numbers in normal blood; the second, the most common form, in lead poisoning and pernicious anæmia; the last in those cases of lead poisoning in which nucleated reds are plentiful. These last granules, in position and size, suggest a breaking-down normoblast nucleus. The reds are otherwise normal. Others deny that transitional stages between fragmenting nuclei and these occur, and point out that they are least in the bone-marrow where karyorrhexis is most common. Again, it is claimed that they do not occur until definite signs of regeneration have also occurred (but the hydræmia is also at its maximum then), well seen in post-hemorrhagic anæmia, hence the opinion of some that they are related to regeneration. In favor of this is the difficulty of producing them by the direct influence of lead salts.

Cadwalader[25] finds them always associated with nucleated reds in lead poisoning, and thinks them the result of a fragmentation of the nucleus of the red cell. In favor of this is the fact that the increase in nucleated reds precedes that of the granulated cells, and he thinks the forms of granules suggest steps in the process.

At this point we wish to state that those dealing with these granules do not exclude any other basophile granules, hence practically every granule found in red blood-cells is described under this one title. In our opinion there are at

[22] Hamel, Deutsch. Arch. f. klin. Med., May 23, 1900.
[23] Am. Jour. Med. Sci., September, 1901.
[24] Am. Jour. Med. Sci., May, 1902.
[25] Bull. of the Ayer Clin. Lab., Univ. of Pa., January, 1905.

least three different basophilic granulations in red blood-cells, that these when compared side by side have little resemblance the one to the others, and, we suspect, no relationship; but the scope of this book could include a discussion of the latter point only in so far as it emphasizes their appearance or occurrence.

The malarial granules are described on page 446. Compare them side by side with the Grawitz granules, and they do not seem to belong to the same class of structures. Grawitz believed them different, but did not state reasons. The one cannot be seen in the fresh cell, the other can.

The granules described by Vaughan (see page 401) as remnants of nuclei do not resemble the Grawitz granules, and yet from Cadwalader's figures we judge he includes them as his coarse variety. It is impossible to say they are not related, but they do not look as if they were.

Perhaps their greatest importance is in lead poisoning, since here they may be the only abnormal blood-feature. In other diseases in which they occur, as the anæmias, they form but a minor part of the blood picture, although they occur in large numbers. They are very fine in size. Some claim that they can be found in the blood of all lead-workers. This may be the case, but it depends on the length of time that the specimen is studied, and we do find cases of lead poisoning, particularly the peripheral neuritis cases, the one condition in which it would be most important to find them, in which they have not been found, or only one cell, in the time at the disposal of the ordinary clinical worker. They are especially numerous in cases with gastro-intestinal features, to which symptoms they bear a rough parallelism, but this may be better explained by the fact that both are early features of lead poisoning. They vary much in number from day to day. As a rule they appear very early, even after four days' exposure to lead, and they may be present in the blood of those exposed over twenty years. They are the first sign of the anæmic blood changes, and the last sign to disappear, hence we may speak of an anæmia even before the count drops. In the diagnosis of intestinal colic they may be of importance, but in the cases of peripheral neuritis we have failed to find them so.

Other references to this subject are, Naegeli,[26] who considers them related to blood regeneration; Boellke,[27] who denies that they bear any relation to the nucleus.

Number of Red Blood-Cells.—The average count for the normal adult man is usually given as 5,000,000 cells per cubic millimetre of blood; for the woman, 4,500,000. In a healthy young man, however, it is more common to find from 5,000,000 to 6,000,000.

By *polycythæmia* is meant a condition with more cells per cubic millimetre than this present; by *oligocythæmia*, one with a smaller number. It is evident that this number is simply relative, that variations may be due either to actual variations in the number of red blood-cells in the body, or to the amount of plasma, which may by diluting the blood cause an oligocythæmia, and when it is reduced in amount, a polycythæmia.

The blood-count may vary in different parts of the body. Oliver [28] found that anything which increases the blood-pressure even locally will cause a rise in the count at that point; as, for instance, in a limb that has been hanging in a dependent position, active or passive motion,

[26] Münch. med. Wochenschr., 1904, No. 5.
[27] Virch. Arch., 1904, vol. clxxvi. S. 47.
[28] Brit. Med. Jour., 1896.

digestion, etc. These variations are, however, quite slight; yet exercise will raise the count, the local application of cold and of heat lower it or raise it, according to the production of stasis, vasodilatation, or constriction.

Excessive exercise, Willebrand found, would raise the count of red cells from 3 to 23 per cent. (average 12.3 per cent.), and of leucocytes from 19 to 97 per cent. (average 47 per cent.).

Physiological Variations.—The effect of *sex* has already been mentioned. This variation occurs only during the menstrual period of life, since for girls until their fifteenth year the count averages 5,444,000, while for boys of the same age 5,102,000; between the ages of forty and sixty, again the count of women averages 5,000,000.

The count varies much with *age*. The maximum is at birth, in which case it may be even 7,000,000, but, as a rule, is lower,—*e.g.*, 5,740,000 (Stengel and White). Otto found the average for the first four days to be 6,155,000; in one child ten hours old, 6,910,000. It depends somewhat on the time at which the umbilical cord is tied, since by tying late there may be a gain of almost one million cells per cubic millimetre. After the first four days the count begins to drop, and is at a minimum in about one year. These high counts at birth are probably due to the concentration of the blood; the body is not yet accustomed to its environment, and loses considerable water. They last but a few days, not over ten, after which nucleated reds also disappear.

From birth until about the tenth year the count reaches the minimum, then slowly rises. There is considerable difference of opinion when this minimum occurs. Gundobin gives the average count during this period as 5,100,000. It rises from puberty to thirty years of age, during which period young healthy persons often have from 5,500,000 to 6,000,000 cells. From about thirty to fifty years, 5,000,000 for men, 4,500,000 for women, may be considered normal, and after forty the count is inclined to slowly drop in men and rise in women.

Not satisfied with the age curves usually quoted in text-books, we have attempted one, using the material of this clinic, especially the neurasthenics and a few patients with apparently normal blood. In addition to this we have the very valuable studies of our medical students on their own blood, counts made to conform to the most rigid criteria of accuracy (see page 413), as accurate, we believe, as any which have yet been published.

We have used means, not averages; this is, we think, the correct way of arriving at a fair estimate of blood-counts, etc., for the extremes should not be considered when the question is of the most common condition. All the figures are arranged in order of magnitude, and that chosen as mean around which the greatest number clusters. For a discussion of the low hæmoglobin estimations, see page 465.

Blood of Patients.

MALES.

Years.	Cases.	Reds (mean).	Hb mean Per cent.	Index.	Leucocytes.
6 to 15	5	5,560,000	85	....	7500
16 to 25	36	5,200,000	85	0.8	6500
26 to 35	69	5,300,000	90	0.85	7000
36 to 45	42	5,500,000	90	0.82	5500
46 to 55	21	5,300,000	80	0.75	9000
56 to 65	9	5,000,000	80	0.8	....
66 and over	5	4,000,000	60	0.77	7500

FEMALES.

Years.	Cases.	Reds (mean).	Hb mean Per cent.	Index.	Leucocytes.
10 to 15	5	5,000,000	75	0.75	8000
16 to 25	43	4,500,000	77	0.85	7500
26 to 35	55	4,500,000	80	0.88	7200
36 to 45	34	4,600,000	72	0.80	7700
46 to 55	17	4,500,000	77	0.85	7000
56 to 65	10	4,500,000	70	0.78	6000
66 and over	3	4,700,000	65	0.7	7000

The students' counts showed the following: age, twenty to twenty-five years; males, 176 cases; mean of reds, 5,000,000 (extremes 4,500,000 and 6,700,000); 14 (8 per cent.) were below 5,000,000, and 15 (8.5 per cent.) above 6,000,000); of leucocytes, 7500 (52 cases); of hæmoglobin, 14.5 gms. (Miescher), 92 per cent. (Fleischl), 95 per cent. (Dare), 92 per cent. (Gowers).

Females, 16 cases; mean of reds, 4,800,000; of leucocytes, 8000; of hæmoglobin, 11 gms. (Miescher), 85 per cent. (Fleischl), 87 per cent. (Dare), 82 per cent. (Gowers).

Nutritional Conditions.—In thin muscular persons the count is somewhat higher than in the stout. A large meal may cause a temporary slight decrease, said to be due to the increased fluid of the plasma. During hunger periods there is an increase, a rise of a half-million cells in twenty-four hours being common, attributed to concentration of the blood.

The *temperature* has an influence on the count. In winter there are about 500,000 more cells per cubic millimetre than in summer (this was well seen in some of our students' counts). The change of residence from temperate zones to the Tropics may lead to a drop in the count of from 500,000 to 2,000,000 cells.

Pregnancy.—For both mother and the fœtus there is said to be a diminution in the count during the last part of pregnancy; for the mother a drop of about half a million cells and 20 per cent. of hæmoglobin; for the fœtus of from seven and a half to eight and a half months the count was found to be 7,000,000, while at nine months 6,500,000 (Biondi and Gardini). The blood of mother and child are, on the whole, rather independent; in case the mother has an anæmia-producing disease the child can preserve its count fairly well, and *vice versa.*

Thompson made a very careful study of twelve cases in Dr. Williams's clinic of this hospital. He found a moderate decrease in the reds from the fourth to the eighth month. The count and hæmoglobin rise to normal at term. The specific gravity shows the most striking curve parallel to that of reds and hæmoglobin, but more accentuated, with the initial fall and terminal rise, the minimum (1040.8) at the sixth month.

Altitude.—Very much work has been done to decide this, one of the most interesting and hotly debated chapters of hæmatology, since the rise in count in persons ascending to high altitudes is a phenomenon long ago witnessed, one concerning which there is no doubt, and yet with its explanation not yet wholly clear. The count increases as persons ascend at the rate of about 50,000 cells per one thousand feet, and diminishes as soon as they descend, or at the latest in thirty-six hours. The increase in the count is more marked the more sudden and higher the ascent; there is little from an ascent of 1200 metres, slight and tardy if 1800 metres, but at once if 3000 metres. The rise is certainly more rapid than could be explained by a new formation of blood, and on the descent with the decrease there are no signs of blood destruction. The rise is best seen in invalids, especially those with lung tuberculosis. The symptoms of anæmia are even aggravated.

There would now seem to be two factors which enter into the case,—the first a temporary one, due to a changed distribution of the blood-cells, and later, in eight or ten days, a permanent change due to a true new formation of cells.

Miescher and his pupils consider that the diminished oxygen tension is the stimulus to new blood formation, confirmed by the work done in many laboratories and by many men, Jaquet *et al.* Evidence of an increase of blood was found in animals kept for several days in an atmosphere of reduced tension, or by keeping these animals at high altitudes. And yet practically all of this experimental work has been challenged, and animals sent to high altitudes give disappointing results.

Other explanations were given:—that there was a concentration of the blood due to evaporation (Grawitz), hardly possible, since the solids of the plasma do not change as much as the count; that it was an accumulation of cells in the capillaries (Tuntz); that the cells lived longer; that there was an initial fragmentation of the reds causing the early increase, and then a true new formation (Koppe); that there was a peripheral vasoconstriction causing a concentration of the blood from the increased lymph formation (Bunge); and lastly, that it was due to the error in the instrument, the reduced atmospheric pressure affecting in some inexplicable manner the thickness of the stratum of blood counted (Gottstein). So many papers have recently appeared on this subject that we shall mention but a few, especially that of Campbell and Hoagland,[29] who consider that the change is due simply to a changed distribution in the blood-cells, depending on the lowered blood-pressure, this due to the lowered barometric pressure, and to a compensatory increased heart action, hence the pulse increases almost parallel to the count. Mosso showed peripheral vasodilatation, hence stasis in dilated capillaries, at altitudes. The heart will compensate all these factors if the person remains at an altitude, hence later the count returns to normal. The difference in temperature has some influence; hence the count at Colorado Springs is about 800,000 lower than at the city of Mexico, two places of the same elevation. Some consider this the chief factor (Weinzirl, *et al.*). Experiments with rabbits showed a decreased count in the mesenteric circulation corresponding to the rise in the peripheral circulation.

[29] Am. Jour. Med. Sci., November, 1901.

The experiment of Gaule, who studied his blood during a balloon ascension and found the rise accompanied by the appearance of many nucleated reds, has not been confirmed.

Drugs and Therapeutic Measures.—Among the drugs which increase the count by their effect on the reds are iron, which is almost a specific in chlorosis, affecting especially the formation of hæmoglobin, and arsenic, a drug equally valuable in pernicious anæmia, which seems to affect the production of the red blood-cells. Mercury in large doses causes an anæmia. The destruction of weakened cells by this drug may explain the Justus test for lues. Lead causes a chlorotic anæmia, explained by some by the gastritis, by others by its direct injury of the red blood-cells. In favor of the latter view is the granular degeneration so constant in these cases.

Any drugs which affect the amount of plasma by causing rapid losses of fluid to the body, as diuretics, emetics, purgatives, diaphoretics, will cause a rise in the count, providing the change be sufficiently rapid. Yet one is usually disappointed in the slight effect of these drugs.

Cold baths cause an average increase of 1,860,000 (Thayer). This is due to the peripheral vasomotor constriction, hence stasis in the capillaries. The specific gravity also is increased. The maximum increase is immediate, and disappears in about one hour. Breitenstein thinks the effect of a cold bath is greater for a typhoid patient than for a normal man, since the distribution of cells is already abnormal.

There is often a transitory post-operative rise of 100,000 to 1,000,000 cells, probably a peripheral phenomenon.

Pathological Conditions.—The acute cachexias of infectious disease, due to the toxins of certain of the specific fevers, can cause a marked anæmia, even when there are no hemorrhages. This is true in certain cases of pneumonia and of typhoid fever, but is by no means common. With the lowering of the count there is increased pigment in the urine and increased globulicidal properties of the serum. Hence there is probably an increased destruction of the red cells.

Chronic Cachexia.—Of this tuberculosis, cancer, and lues are the best illustrations. (See pages 562, 576, and 581.)

The methæmoglobin-producing poisons diminish the count, because of their direct destruction of the cells. Among these are pyrogallic acid, the chlorates, certain of the coal-tar products, as antifebrin and phenacetin.

Other pathological conditions raise the blood-count, as phosphorus poisoning, especially acute cases, in which the count has risen in two or three days even to 8,650,000. Since the solids of the plasma are not correspondingly increased this rise is not due to globular concentration, and in some cases there is no vomiting at all. The cause is

uncertain. The same is true of carbon monoxide poisoning, in which counts of 6,630,000 have been reported in cases without vomiting.

In cyanosis, particularly that due to congenital heart disease in which the count may be from 8,000,000 to 9,000,000, but also to a less degree to lung and other heart troubles, especially mitral, the count may be exceedingly high; or when the color is extreme the count may be normal. In local cyanosis, that due for instance to hemiplegia, there may be a local rise in the count. In lung disease, especially emphysema, acute miliary tuberculosis, and pneumonia, the count is high; in emphysema and heart disease, even 7,000,000; in adherent pericardium, above 6,000,000; in Reynaud's disease there is a local rise at the affected parts; while in a very interesting group of cases of cyanosis, first reported as an independent disease by Osler,[30] the polycythæmia is extreme, the highest on record, in one case reaching 10,200,000, in another case, 10,000,000. In all these cases the cause is very doubtful. Some explain it as a change in the distribution of the blood, some as a concentration from loss of plasma, others as an over-production of red blood-cells; others say these are longer-lived than normal; while most admit that they do not know.

Osler reviewed nine cases, four of which he reports. The cyanosis was extreme, lasting even for years. The highest count was Cabot's, of 12,000,000, and but one was below 9,000,000 (8,250,000); hæmoglobin, 120 to 150; specific gravity, 1067 to 1080; leucocytes, 4000 to 20,000, but most below 10,000. This publication of Osler's has stimulated a good many reports of cases with high counts. Zamfirescu reports the case of a woman with cyanosis, dyspnœa, and cough; reds, 7,000,000 to 7,300,000; hæmoglobin, 105 to 116; leucocytes, 9000 to 10,000. Kikuchi reports a case with bronchiectasis. Türk[31] reports seven cases like Osler's, two with autopsy, with counts from 7,700,000 to 10,600,000. He suggests that the cyanosis is not due to deficient aëration of the blood, but to a crowding of vessels with an excessive number of cells; that these cases are not rare, but are diagnosed as pseudoleukæmia or hypertrophic liver cirrhosis; that the enlarged spleen is not the primary lesion; that this is a primary hyperplasia and increased function of the erythroblastic myelogenous tissue, hence the disease is analogous to leukæmia, except that there the hyperplasia is of the leukoblastic marrow elements; in favor of this is also the constant presence of abnormal red cells and of myelocytes. Other interesting cases of polycythæmia without cyanosis occur, as Zandy's,[32] who proposes the term "erythrocytosis." His was also a case of splenomegaly; the count was even 9,500,000. Türk[33] reports a case with cirrhosis of the liver and enlarged spleen, with a count of 9,300,000; leucocytes, 37,000; hæmoglobin, 18.2 gms. Gresböck[34] mentions cases with nothing but the high count.

The counts locally high are especially important, for one counting blood must exclude them by not taking his drop from a blue ear or finger.

[30] Am. Jour. Med. Sci., 1903, vol. cxxxvi.
[31] Wien. klin. Wochenschr., 1904, Nos. 6 and 7.
[32] Münch. med. Wochenschr., 1904, No. 27.
[33] Deutsch. med. Wochenschr., 1904, No. 50.
[34] Deutsch. med. Wochenschr., 1904, No. 20.

There is one point to be borne in mind in this connection, that we usually count capillary blood, not arterial or venous, and the count in the capillaries need not be the same as that in the vessels. A capillary field of the frog's mesentery or rabbit's ear, *e.g.*, is seen to contain few corpuscles moving in single file through the capillaries, and some channels so narrow that the cells do not enter them at all. In cases of active congestion from warmth, or of venous stasis, the capillary bed is much widened, the capillaries filled with cells, even those which before transmitted only plasma; hence the count is higher. It is not so much changes in the relation between plasma and tissue lymph which can cause very rapid changes in the capillary count while that in the arteries may remain constant, as factors governing filling and circulation of the capillary area. The count in capillaries and veins is about the same. Following more marked changes in temperature, as *e.g.*, after a cold bath, the count does rise in the arteries, since then the flow to the tissues is increased.

These changes are much more marked in the leucocytes than in the reds, since the former collect in the vessels, forming layers along the walls.

Cyanosis may deceive one much, as, for instance, in a case with normal blood-count which at autopsy shows a condition suggesting pernicious anæmia. The same is true of certain dysenteries.

Similar cases with high counts follow the use of various coal-tar products.

A student recently handling aniline oil became cyanotic; red cells, 5.900,000; hæmoglobin, 107 per cent. (Dare); leucocytes, 6100. Six days later the reds were 5,084,000, hæmoglobin, 78 per cent. (Dare).

Resistance of the Red Blood-Cells.—Many methods have been proposed for determining the resistance of the red blood-cells, in the hope of explaining phenomena such as hæmoglobinæmia. At first these methods were mechanical, chemical, electrical, but are now biological, the side-chain theory of Ehrlich being invoked to explain it.

Hamburger's Method.—Sixteen small glasses, each containing 1 cc. of a sodium chloride solution of various strengths, the lowest 0.4 per cent., and each succeeding one 0.03 per cent. higher than the preceding, are used. One drop of blood is placed in each, stirred, allowed to stand for six hours, and then examined to see if it is laked. Normally the lowest concentration which the cells will endure is 0.46 per cent. Since the serum is equivalent to 0.9 NaCl solution, it is evident that the cells live in a hypertonic medium. With this method the resistance has been found increased in anæmias, in hæmoglobinæmia, after many poisons, in typhoid fever, erysipelas, pneumonia, and jaundice. The method is faulty, however, since in paroxysmal hæmoglobinuria the cells have been found normal, but of lowered resistance to mechanical disturbance. Stengel proposes the following method. The blood is diluted 1 : 10 in a Zeiss leucocyte pipette, with sodium chloride solutions varying from 0.42 to 0.52 per cent. The blood is mixed and then blown into small tubes sealed at one end. These are allowed to stand and then centrifugalized for from two to five minutes, then held against a

white paper to see in which the corpuscles have laked. The advantage of this method is that more fluid is used, and that the percentage is not materially altered by the salts which escape from the cells. Stengel considers it is not osmosis alone, but a chemical or a vital reaction between the stroma and the Hb, which holds them together. As a result of his experiments he found that saturation with an excess of carbon dioxide (as in congestion) causes no great morphological change and no particular alteration in the cells, and yet the vulnerability is greater; that cold produced marked changes, as, for instance, if the congested finger be frozen, which is suggestive concerning hæmoglobinuria; that hypotonic salt solutions have the power in the test-tube as well as within the blood-vessels of decolorizing and vacuolating red blood-corpuscles. This point is disputed by many workers, who find that even the intravenous injection of distilled water does not lake any blood-cells. Heat of even slight degree changes the shape and size of the corpuscles and finally decolorizes them. A higher degree causes budding, vacuolation, and a somewhat higher degree complete fragmentation (see page 396).

Mechanical Influences.—In some conditions the cells have been found to have varying resistance to shaking. Meltzer has shown[35] that the effect of shaking depends upon the rapidity of vibration, that for each blood there is a minimum and maximum rate which the cells can bear without destruction. Laker has tested the cells by passing the discharges of a Leyden jar through them. Various other methods have been proposed, but with as yet little result.

The Estimation of Hæmoglobin.—This should be the easiest and the most useful determination in blood-work, and it is unfortunate that the use of faulty instruments has resulted in the accumulation of a vast amount of data of very little value; for the estimation of hæmoglobin is of more importance than the blood-count, as it is so much less time-consuming. In the case of the blood-count we know, supposing the work has been neatly done, what is meant by the figure given; but for hæmoglobin we must know the kind of instrument used, its make, and the amount it has deteriorated. In general we know remarkably little that is accurate about the hæmoglobin content of the blood in disease.

It is unfortunate that instruments do not all read in grammes rather than percentage, for their makers do not agree what quantity of hæmoglobin should be called normal, nor is there a figure which is normal for all ages, since the age curve of hæmoglobin is very hilly. If instruments are to read percentages, it would be well to have one for each of the various periods of life, since the blood of a normal child of about ten years would read but 80 per cent. on an instrument standardized for a normal man of thirty years. Were hæmoglobin expressed in grammes, there would be less danger of calling such a child "slightly anæmic;" it would also be easier to restandardize an instrument.

Another element of error is that instruments are standardized against hæmoglobin in dilute water solution, while hæmoglobin in an albuminous fluid like the blood-plasma will give higher readings; hence in reading the blood of extreme anæmias we may get misleading figures. For instance, in post-hemorrhagic anæmia the hæmoglobin would seem to drop much lower than is really the case.

In judging the various instruments, one should consider the principle on which the instrument is constructed, and whether or not the maker has followed this principle well. For instance, the v. Fleischl seems well made, but there are several inaccuracies in its principle; the Gowers instrument has fewer inaccuracies, but is so poorly made by many manufacturers that it has fallen into disrepute.

[35] Johns Hopkins Hosp. Rep., vol. ix.

Miescher's Modification of the Fleischl Instrument (see Fig. 105).—This is at present our best instrument. It is, however, for laboratory use only, since it is expensive, bulky, and requires a dark room, considerable time for each determination, and considerable practice. The blood is mixed in a beautifully made pipette (see Fig. 106), which allows dilutions of 1 : 200, 1 : 300, or 1 : 400. The markings of these pipettes are particularly good, especially the small lines on either side of each main line, each indicating $^1/_{100}$ of the length of the column between the tip and the "1" mark, thus obviating the necessity of losing valuable time in trying to bring the blood column exactly to a mark. The polished conical end is also an advantage.

A large drop of blood is aspirated to the point indicated for the desired dilution. Such a dilution should be chosen as will allow the

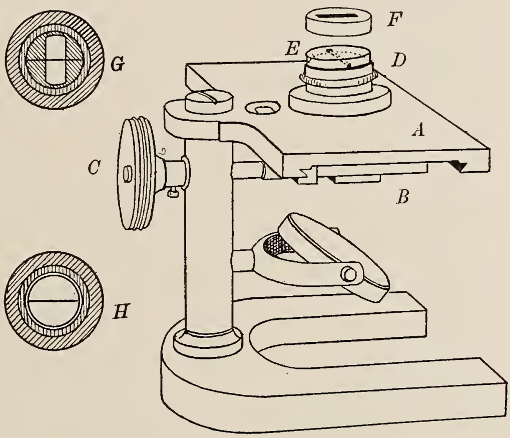


FIG. 105.—Miescher's modification of Fleischl's hæmoglobinometer. *A*, stage; *B*, color-prism rack; *C*, milled head; *D*, cell; *E*, cover-glass; *F*, cap; *G*, cell seen from above; *H*, cell of Fleischl's instrument.

readings to be made near the middle of the color-prism. A normal blood cannot be diluted to the 1 : 200 mark, since the readings will be "off the scale" of the table of equivalents. The diluting fluid used is 0.1 per cent. sodium carbonate. (A stock solution of 10 per cent. is diluted one hundred times.) Distilled water has been recommended, but with the dilute soda solution we get a color-tone more approximating that of the prism, which materially aids the readings. The blood is mixed exactly as for a blood-count, is well shaken, then the contents of the capillary tube are blown out. In filling the cells (of which there are two, one 15 mm. and the other 12 mm. in depth) it is essential first to fill one side with water, to make sure that there is no leakage into the other half. The blood, well shaken, is blown into the other chamber of the cell. Both the water side and the blood side should have convex meniscuses. The cover-glass, E, is then slid on

carefully, pushing off this excess and leaving the chambers exactly full. The slightly raised partition prevents mixing. The small cap, *F*, is then put in place, to hold the cover-glass secure and also to limit the field of vision to one about 3° in length. The cell is then placed in the receptacle on the stand, *A*, and the instrument placed in a screen which admits the light at one point only, where it will fall directly on the mirror and illumine both sides equally. The light to be used is a yellow flame, whether from gas, oil, or candle. Electric light, a gas-light with a mantle, or sunlight, cannot be used. The person should sit in a comfortable manner with the eyes about 25 cm. above the instrument, and make his readings with both eyes open. The milled head, *C*, moving the color-prism, is then rotated until that part of the prism (see Fig. 107) which just matches the color of the blood-mixture is under the water half of the cell. In doing this it is well to make quick excursions to both sides of the point, gradually diminishing them until the point of matching is reached. Since the retina is soon fatigued, and is not then sensitive to colors, the eyes should be rested after each fifteen seconds of color-matching. A very conscientious, painstaking student will sometimes get results much worse than the careless student, since through careful work the eyes are fatigued. When the color is matched the reading is made. At least five such readings should be taken,—ten are better,—and the mean, not the average, used. The blood is then removed by sucking it up with the mélangeur from this (the deep) chamber, the shallow chamber filled in the same way, and a similar series of readings is made. Since these cells have heights which are to each other as 5 is to 4, and since different parts of the color-prism are used, if the readings of the lower multiplied by $^5/_4$ differ from the average made with the higher by not over 2 per cent., and the instrument is a well standardized one, it is seen that the readings are so controlled that considerable error at this point is impossible. We insist that the student shall if necessary put the blood back again into the deep chamber and repeat the work until the readings with the two cells, both calculated for the 15 mm. cell, do not vary over two points.

Fig. 106.—Mixing pipette of Miescher's hæmoglobinometer.

The great advantage of the instrument is that each is accompanied by a scale which gives the number of milligrammes of hæmoglobin per litre of diluted blood corresponding to the readings of that particular instrument. It is of the utmost importance that the right book be used. It is then easy, making due allowance for the dilution, to

determine the number of grammes of hæmoglobin in 100 cc. of blood, the desired result. If then, with due observance to the age curve, the worker wishes to express his answer as a percentage, he is at liberty so to do. This instrument has been found to be correct within 0.2 per cent. of hæmoglobin. In case a light screen is not at hand, a tube of blotting-paper may be fitted over the cell, thus the side light

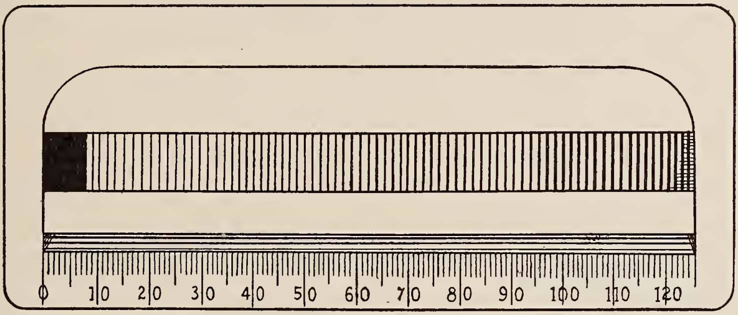


FIG. 107.—Color-prism of the Fleischl-Miescher instruments.

is screened from the eye over this tube. A person should be careful in this case to change frequently the eyes, so that neither may become fatigued.

The mixing pipette is cleaned, etc., just as is that used in blood-counting.

Fleischl Hæmoglobinometer.—This, until within a few years, was in this country the favorite instrument. The Miescher machine just described is an improved form of this. The blood is taken in a small short cylindrical capillary tube (see Fig. 108), which is only a few millimetres long, perhaps 1 mm. wide, and holds from about 5 to 8 cmm. of blood. It is fastened in a small metal holder by means of cement. To wash this in alcohol or ether results invariably in loosening the tube, and when once loosened it is so small and easily broken that the pipette is as good as ruined. It should be cleaned by water, and then by drawing through it a needle with a thread soaked in alcohol and then in ether. It must be perfectly dry. The point of prime importance is that the number on the handle of this pipette shall correspond to the number on the post of the machine, otherwise gross errors will certainly result. This pipette is filled by touching its end to a large drop of blood. It should not be stuck into the blood, since any wetting of the outside is to be avoided. One makes sure that there is no blood on the outside, and that the tube is exactly filled, having neither a concave nor a convex meniscus. Meanwhile,


FIG. 108.—Pipette of the Fleischl instrument.

the cell (see Fig. 105) of the instrument has been filled on the one side with water and on the other side by a few drops of water. The pipette is dropped into this latter and emptied by rapidly agitating it in this water; then with a few drops of fresh water the drop of fluid clinging to it is washed back into the cell. By means of the handle of the pipette the blood is then thoroughly mixed with more water, until this chamber of the cell is filled to the brim. The two halves should then be exactly full, without concave or convex meniscus, and certainly without any leakage from one side to the other. They may be covered over with a suitable cover-glass. In a dark room the instrument is read as the Miescher.

There are a few precautions to observe. The images of the two chambers should fall on the right and left halves of the retina, never on the upper and lower, since the lower half of the retina is not nearly so sensitive as is the upper; the light should never be in front of the instrument, but at the side; as small a candle as possible should be used; if there is no screen handy, a tube of dark paper will suffice to cut out extraneous rays. The inconveniences of the machine are the following: in the first place, one is looking at a color field of the prism, which varies at its extremities by at least 15°, and the observer must try to read the color at the centre of a field with such wide variation as this (compare the cells *H* of the Fleischl with *G* of the Miescher). It is difficult to see how a person can claim to make readings within 2 per cent. Again, the instrument has certainly not been standardized as accurately as is desirable, there being considerable difference between the older and the newer instruments, and even in the latter it is stated that the prism has straight sides, which certainly does not fulfil the requirements of a good optical instrument, since depth of color is not directly proportional to thickness of glass. For this reason all readings should be made on the upper half of the prism; hence, if the blood be known to be anæmic at least two or three pipettefuls are employed for each determination. The instrument is bulky; it is also expensive. The greatest objection to it that we find is that it has the appearance of accuracy without the reality. A person that has used no other machine is usually confident that he can read within at least 2 per cent. We suspect that the error inherent in the machine is at least 5 per cent. The observer should be very careful to use his retina not over fifteen seconds at a time, to prevent fatigue. To clear the blood in case of lipæmia and high leucocytosis by means of ether and potassium hydroxide is not to be recommended.

Gowers' Instrument.—This little instrument (see Fig. 109) is much to be recommended, perhaps is the best for the general practitioner. It is cheap, easily portable, simple, and should be fairly accurate. It consists of a color-tube, B, containing a fluid the tint of one per cent. hæmoglobin solution, and a graduated test-tube, A, into which 20 cmm. measured in the pipette, C, are diluted with water until the tints match. The percentage is read directly on the graduated scale from the height of the diluted blood.

The blood is drawn to the proper mark in a measuring pipette, and then blown into the graduated tube, in which have previously been placed a few drops of distilled water. By sucking this water

back and forth the inside of the pipette may be quite thoroughly cleansed of the blood, but it should then be again filled with distilled water and this added to wash out the last trace. The blood is mixed with the water by covering the end of the tube with the thumb and inverting it several times, but the thumb should be wiped across the top of the tube, that the clinging drop of water may not be lost to the mixture. It is well in reading these two tubes to use both direct and transmitted light. They are best held against a sheet of white

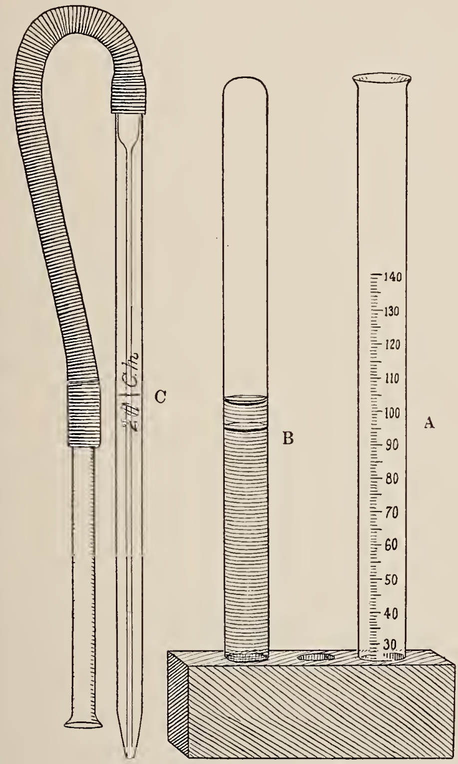


FIG. 109.—Gowers's hæmoglobinometer. A, graduated tube; B, color-tube; C, pipette.

FIG. 110.—Sahli's hæmometer.

paper, and it is also well to cover their upper ends by another piece of paper, that the reader may not be biased by the height of the column. In certain instruments there is a tube for daylight and one for artificial light.

The instrument is not claimed to be accurate within less than about 5 per cent. In buying this instrument it is very essential to get one made by a responsible firm, for the market has simply been flooded by cheap instruments which have not a semblance of accuracy. We recommend those which bear Sahli's name, since he guarantees their

accuracy. It should be remembered that these color-tubes of gelatin stained with picrocarmine certainly bleach, and should be renewed from time to time. When not in use they should be protected from sunlight. An advantage of these instruments is that no greater accuracy is claimed than they possess, and one is not tempted to read to 1 per cent. Another advantage is that there is but one color to standardize instead of a whole scale.

The Hæmometer of Sahli (see Fig. 110).—While this has not been sufficiently tested, it promises to be one of the best. The principle of this little instrument is that of the Gowers, but the color-tube contains acid hæmatin, 1 per cent. solution, and the hæmoglobin of the blood is also, by means of hydrochloric acid, changed to acid hæmatin, a pigment quite constant in composition and color value. The instrument may be used in any light, since the two tubes contain the same substance, and would therefore be modified equally. The blood is obtained in the same way as for the Gowers, and blown and washed into the graduated test-tube into which has already been placed up to the 10 per cent. point a tenth-normal solution of hydrochloric acid. (This may be made with sufficient accuracy by diluting 15 cc. of the pure acid to one litre with distilled water. Sahli recommends that a little chloroform be kept in this stock bottle.) The hydrochloric acid in a few minutes changes the hæmoglobin to acid hæmatin. It is then diluted with distilled water until its tint corresponds to that of the standard color-tube. These tubes are placed in a very convenient little stand with a ground glass back, which renders the reading easy and quite accurate. The color-tube is said not to deteriorate with age so much as that of the Gowers instrument.

Dare's Hæmoglobinometer.—This instrument (see Fig. 111), recently put on the market, certainly promised very good results. A film of undiluted blood is compared with a color-prism stained with golden purple. The pipette (see Fig. 112) consists of two plates of glass, one white, A, one clear, B, between which is a slit of known width. A rather large drop of blood is necessary and will at once by capillarity fill the slit. The pipette is then slipped into the instrument, Fig. 111, B, and the reading made at once by the light of a candle, E, attached to the instrument, not necessarily, however, in a dark room, providing the observer faces a black background. On the instrument is a telescope tube, A, which allows accurate focussing and also an advantageous magnification of the two color-fields,—that of the blood and of the color-prism. By means of a small wheel, D, the prism is rotated until the colors match, and then the reading is made at the knife edge on the edge of the disk. The same precaution of not tiring the eyes is to be observed in this as in all other color-tests. The advantages of the instrument are that undiluted blood is used; that

it is rapid; that leucocytes do not affect the reading as in the other instruments; and that it can be used in a light room. Its disadvantages are that certain of the instruments were not well standardized when put on the market, and while they may now be, still the readings are generally rather low; the instrument is expensive; the readings must be made at once before clotting, since in a very few minutes the reading jumps from 5 to 10 per cent. It is rather a fragile instrument and does not stand the wear and tear of hard usage. Nevertheless, it is rapidly cleaned, it is a very convenient, satisfactory, and, when well standardized, accurate instrument. We do not recommend it as superior to the Miescher, and we do not think it enough

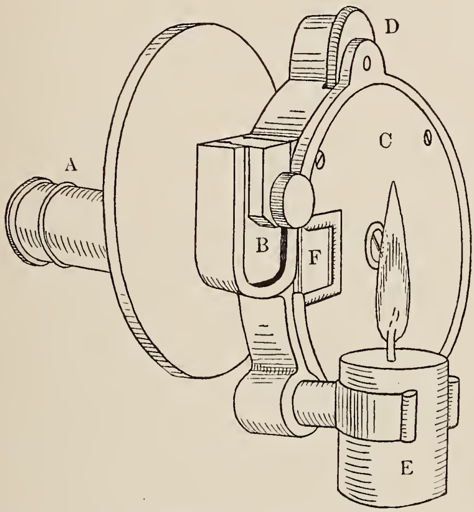


FIG. 111.— Dare's hæmoglobinometer. A, telescope; B, pipette in place; C, case inclosing color-prism; D, milled head moving prism; E, candle; F, window admitting light to color-prism.

FIG. 112. — Pipette of Dare's instrument. A, the white glass; B, clear glass disk.

better than the cheaper instruments, especially the new Sahli, to justify its high price. We have been very much pleased, putting several instruments in the hands of as many workers, to find how closely they agree in their readings on the same case.

The Oliver Instrument.—This instrument is historically important, since it was the first accurate instrument proposed to replace the v. Fleischl, which before then had been accepted as the standard. It consists of two frames of tinted glasses, six disks in each, the colors of these glass disks varying by 10 per cent. The blood is obtained in a large automatically filling pipette, is mixed in a cell with white base, and covered with a cover-glass. By candle-light the color of this mixture is compared with the various glasses until it is decided between which two it stands. The eye must be screened by means of a camera lucida from side rays. By means of riders of colored glass, which raise the percentage 5 per cent. (certain special instruments have a rider for each per cent.), closer readings may be made. The instrument is well standardized and well made. While formerly

it was more accurate than any on the market, it has lately been displaced by the Miescher. Many find it is rather inconvenient to use.

The Tallqvist Scale.—This simple little device was exploited as a great boon to the general practitioner. It is a little book of blotting-paper and a scale of colors. A drop of blood on the blotting-paper is in direct sunlight matched against this color-scale. The instrument costs but a dollar and a half, can be carried easily in the pocket, and a determination made in less than a minute. The colors of the scale vary by 10 per cent., therefore any intermediate percentage must be estimated by the eye. We admit that the trained eye, that is, an eye trained to use more accurate instruments like the Miescher, will soon use this color-scale more accurately than an untrained eye will use the Miescher; but the former can guess as near, even from the color of the lips, without the Tallqvist scale, while of one eminent Viennese it is said that he could guess within 0.5 per cent. from a stain on cloth. This was guess-work, but almost as much so is the Tallqvist method. The instrument was standardized against the v. Fleischl, which does not recommend it.

The spot of blood is obtained by holding the edge of the paper against a large drop which soaks up into the paper. This is then blotted by squeezing it between two pages of the book until the lustre is lost, and the reading is made at once before the drop becomes dry. In comparing the tints, position is of great importance. The observer, facing the sunlight, holds the book rather low before him, so that light is well reflected from the color-scale. A moist unstained ring around the drop is seen in anæmia. Leukæmic blood is poorly absorbed, and has a color not agreeing with the scale. So great has been the demand for these books, following their recommendation in a very excellent text-book, that many editions have been published. There is now a slight reaction setting in against this scale. The personal element enters in too largely, as two persons can differ widely in their opinion, and the trained eye sometimes even judges the tints of the scale, and reads without it. We hope the reaction will lead the practitioners to adopt some instrument requiring less guess-work.

In hospital work we find it a great advantage to have several varieties of hæmoglobinometers in use, and to insist that the student shall use them all. Only in this way will he appreciate the strong and weak points of each. The man who uses but one soon places undue reliance upon its accuracy. Let him use two of different makes and get differences of from 5 to 10 per cent., and he appreciates the need of a standardized instrument.

We have one, a new Miescher, which we reserve as the standard. When a clinical clerk signs for an instrument for ward work, he must first standardize the instrument against this Miescher, and in future work make the necessary correction. Among our forty instruments of seven different types we find that the corrections necessary vary from 1 10 per cent., and are especially large in those over two or three years of age.

These instruments very seldom read 100 per cent. in a normal person. From the records of 176 medical students, all normal men during the third decade of life, the readings made with great care, the Fleischl instruments in 161 cases read from 65 to 110 per cent.; 136 varied from 80 to 100 per cent., and 52 from 90 to 95 per cent.; the mean was 92.5 per cent. Of 156 records with the Dare instrument, varying from 65 to 110 per cent., the blood of 105 read from 90 to 100 per cent.; the mean 95 per cent. Of 150 students using the Gowers, the records varied from 70 to 120 per cent.; 81 stood between 90 and 100 per cent.; the mean about 92 per cent. Here the direct readings of the instruments not corrected with the Miescher are given.

With the Miescher the blood of 125 students varied from 11.4 to 17.6 gms. per 100 cc. (The estimations were controlled for the most part by determinations at the same hour of the following day.) It is much harder or even impossible to state the mean, so uniform was the distribution. The average was about 14.5 gms. Considering this 100 per cent., the blood of 38 students varied from 90 to 100 per cent., and 21 from 100 to 105 per cent. (Note how different the readings on the other instruments.) The variations with the Miescher seemed great, yet they ran more parallel to the blood-counts than did the others. (See page 467.)

In conclusion, the Miescher is a laboratory instrument, and in clinical work is reserved for special cases; then it alone should be used. For the general run of cases without hæmatological interest, either the Sahli, Gowers, Dare, Oliver, or Fleischl is good enough, provided it be a standardized instrument, for it is the scale which is incorrect, and becomes more so with time.

Jolles Ferrometer.—This method, proposed as the most accurate way of determining the amount of hæmoglobin, determines the absolute amount of iron present in the blood. We shall merely outline the method. A small amount of blood is incinerated, the iron of the ash dissolved in a little acid potassium sulphate, and the solution of iron then estimated colorimetrically by means of the ferrometer. In this apparatus the blood-iron solution is placed in one tube, in another tube a standard iron solution. The fluid from this latter tube is allowed to escape through a stopcock, drop by drop, until the tint of the two solutions is the same. For this method a small amount of blood, 0.05 cc., is all that is necessary. The incineration is done in a platinum crucible, the blood being first dried, then incinerated, the potassium sulphate, 0.1 gm., added, and the mixture carefully fused. After it is cooled the ash is then washed into the standard tube. The fluid in the tube used for comparison contains 0.5 mg. of iron and 0.1 gm. of the acid potassium sulphate per cubic millimetre. To each cylinder are then added 1 cc. of dilute hydrochloric acid (1 to 3 of water) and 4 cc. of ammonia sulphocyanide (7.5 gms. per litre). With the apparatus is a table giving the values in hæmoglobin for the various readings.

This instrument is not in use now, for various reasons. In the first place, the hæmoglobinometers are more accurate than they were at the time when this was used. In the second place, it was found that the results with this did not agree well with those of the hæmoglobinometers, and hence a different chemical composition of the hæmoglobin was assumed, or the presence of iron in the blood in other organic compounds. Even this was named "hæmatogen," or hæmatinogen. Both of these arguments are rather lame, since hæmoglobin is a rather constant body, and in the very small amount of blood used the amount of iron in other forms would hardly produce an appreciable error. Perhaps it is the method itself which is at fault. The method has been severely criticised by Pekar,[36] who describes a Winkler method, another quantitative determination of the iron.

[36] Maly's Jahresbericht, vol. xxxiii. p. 241.

Rosin and Jellinek,[37] comparing the Jolles instrument with a Miescher hæmoglobinometer, find no fixed relation between hæmoglobin and total iron. Some cases have high color, diminished iron, a variable count, as uncompensated heart disease. Some have high color, low iron, and normal count, as jaundice, diabetes, Graves's disease. Some have low color and high iron, as some anæmias and chlorosis.[38]

Hæmoglobin.—One hundred cc. of normal blood are usually stated to contain from 13 to 14 gms. of hæmoglobin. Careful estimations of the hæmoglobin at the various ages have shown a regular age curve, quite parallel to that of the red blood-cells, but with variations a little more pronounced.

Age.	Gms. per 100 cc. of Blood.
1 to 4 days	19.329 to 21.160
8 to 14 days	17.869 to 16.124
8 to 20 weeks	15.362 to 12.928
6 months to 5 years	10.971 to 11.373
5 to 15 years	11.151 to 11.796
15 to 25 years	13.034 to 13.870
25 to 45 years	14.727 to 15.013
45 to 60 years	12.484 to 13.150

From this table of Leichtenstern (modified from Sahli's quotation) it is at once evident that the age curve must be considered in all blood-work, and the hæmoglobin given in grammes per 100 cc. rather than in percentage, since there is no one figure which could be considered 100 per cent. for all ages.

By *oligochromæmia* is meant a relative diminution in the amount of hæmoglobin per unit volume of blood. It therefore is seen to have merely a relative and not an absolute value.

By *color index* is meant the percentage of hæmoglobin divided by the percentage of the red blood-cells. This figure, as Duncan first showed, is of considerable importance in some cases. For estimating the denominator, 5,000,000 red blood-corpuscles are considered 100 per cent., while the numerator is the per cent. of hæmoglobin read with any instrument. The color index is less than 1 in all cases in which the blood after anæmia is regenerating, hence in all secondary anæmias, and especially in chlorosis, in which the average is about 0.5, in some cases being as low as 0.3. In pernicious anæmia, on the other hand, the index is increased, the average in a large number of cases of Cabot being 1.04, and one case reaching 1.75 (count 1,000,000, hæmoglobin 35). The high color index is of especial value in differentiating pernicious anæmia from certain cases of cancer of the stomach, a diagnosis clinically hard to make. To be of value, how-

[37] Zeits. f. klin. Med., Bd. 39, p. 109.

[38] See also Mayer, Zeit. f. klin. Med., 1903, vol. xlix. p. 475.

ever, the variations must not be in the second decimal place. It is not a strict mathematical calculation; the figures are too approximate for that.

The question arises at once, What is the color index reckoned in the above manner for the average normal man and woman? Five million is only in very general terms the normal figure, and few hæmoglobinometers read normal blood at 100 per cent. As stated on page 465, taking instruments as they come, the index in fairly normal persons varies from 0.80 to 0.88. In the case of our students, the men, with the Fleischl and Gowers instruments had an index of 0.84, with the Dare, 0.87; the women, with the Fleischl, 0.88, Dare, 0.9, Gowers, 0.82. Yet we often see cases with the above indexes reported as "mild chlorotic anæmia." All our cases are to be judged in the future with this normal index in view.

The question was approached in still another way. Our students' work included fifty-three records of counts and Miescher hæmoglobin estimations made on the same bloods at the same hour. The counts varied from 4,600,000 to 6,700,000, and the hæmoglobin from 10.9 to 17.2 gms. If in each case the number of grammes of hæmoglobin per 1,000,000 cells be reckoned, the mean of these figures should be an approximately normal color-index. These quotients fell within surprisingly close limits, 42 of the 53 varying from 2.2 to 2.8 gms.; mean, 2.63 gms. per 1,000,000 cells. Using this figure as the standard of hæmoglobin content, we hope in the future to be more accurate in our use of the color index.

One point we may be pardoned if we emphasize. Students seem to consider that the mechanism regulating the blood-count is almost as delicate as that controlling the body heat, and that the count of the blood of normal men should vary almost as little as does the temperature, although that varies somewhat. The more carefully the counts are made, the better the instruments used for hæmoglobin, the more evident are the individual, both general and local, the daily and seasonal, and the racial, variations. It is more like the height, weight, or muscular development. And yet the regulation of the composition of the blood is wonderful. It varies within quite narrow limits, although through the vessels pass enormous amounts of water as in diabetes insipidus, of water and solids as in diabetes mellitus, of albumin and water, as is seen in cases of rapidly collecting ascites repeatedly tapped with the frequent withdrawal of even 8 litres of what is practically blood-plasma, while the blood remains wonderfully little changed. Yet no one figure is normal for all men, nor constantly for one man. The same is true of the total amount of hæmoglobin per 100 cc., and also for that per cell. Hence, in judging of blood reports one must not try to apply any hard or fast rules, but to study these variations, since they may be put to practical use.

The "*volume index*," or the quotient of the volume per cent. and the count per cent. (5,000,000 = 100 per cent.) promises well.[39] To determine the volume of the corpuscles Capps uses the hæmatocrit and undiluted blood. A length of the column of corpuscles of 50 per cent. he accepts as normal, hence the 100 per cent. of the calculation (see page 415).

The most important result he obtained is that in pernicious anæmia the color index never exceeds the volume index; that is, that there is no supersaturation of corpuscles with hæmoglobin, and the high color index is due to increase of size alone. Yet on the other hand, in other anæmias the color index may fall below the volume index, and the color index drops more rapidly than the volume, while

[39] Capps, Jour. of Med. Research, 1903, vol. v.

during regeneration the volume returns to normal first. He has never seen any evidence of "acute dropsy" of the cells.

WHITE BLOOD-CELLS

Granulations of Leucocytes.—By granules are here meant the minute bodies, usually spherical, of a size and staining character fairly constant for each granulation, which seem to be not accidental but to belong to the protoplasm; they are perhaps the product of the secretory activity of the cell, but not in the sense that all protoplasm is slightly and indefinitely granular; they are definite inclusions in the cell protoplasm, and are liberated as independent bodies which swim free in the plasma when the cell breaks up. From them can perhaps be distinguished degeneration of protoplasm, accumulations of products of metabolism, and inclusions from phagocytosis.

The various granulations as classified by Ehrlich are, EOSINOPHILIC, ACIDOPHILIC, or OXYPHILIC (α). These granules are coarse, about 1 micron in diameter, spherical or slightly oval, of quite a uniform size and color, very refractive, and hence in the fresh specimen appear black; these from a mixture of stains will always take the acid ingredient. These granules occur in all animals whose blood has been examined from the frog to man. They are of albuminous nature and not fatty as their appearance would indicate. Barker has found that they contain iron.

AMPHOPHILIC (β).—These granules are described as of about the same size as the α, or a little smaller, and yet slightly variable, and as taking both acid and basic dyes. They stain like the eosinophilic granules, except that in a mixture of eosin and indulin they will take the latter; they also take certain basic stains. They occur often in the same cell as the α granules, hence Ehrlich considers them a younger stage of these, and this to be the only case in which two specific granulations are found in one cell. They are found in some leucocytes of the bone-marrow of man and various animals (rabbit and guinea-pig), and in the peripheral blood in certain anæmias. These may explain, in leukæmia for instance, the variations in the size and tint of the granules in some of the eosinophile cells. A few may be present in an eosinophile or all the granules of the cell be these.

BASOPHILIC AND MASTZELL GRANULES (γ).—In the connective tissue and blood of all animals and man are cells which contain large basophile granules. Those of the tissues stain best with dahlia, taking a tone metachromatic rather than strictly basophilic, resembling that of mucin, of which, indeed, some claim that they are composed. This tone is best seen if polychrome methylene blue be used, and is explained as due to the methylene azure of this stain. The granules are spherical or oval in shape, and vary considerably in size in the same cell. Cells

containing somewhat similar granules occur to a small per cent. in normal blood, and are increased in leukæmia, while in some cases of pleural exudate and of gonorrhœa this may be the chief granulation of the leucocytes of the pus. Considerable doubt exists, and is admitted even by Ehrlich, concerning a relationship between the basophile cells of the blood and of the tissues, the latter the true Mastzellen. Their origin seems different. In fact, their only resemblance seems to be that both have basophile granules, yet which do not stain exactly alike. What is more, considerable doubt exists whether the γ granules in cells of abnormal blood are exactly the same as those present in the normal blood and bone-marrow, since even between these certain variations in the staining qualities exist, and those in leukæmic blood are more soluble in aqueous solutions than those of normal blood. It is, therefore, at least possible that we have here to do not with one specific granulation, but with three or more different granulations, or with the same granulation at different stages of its development.

Basophile Granulations (δ).—These granules were originally described by Ehrlich as occurring in the mononuclear cells, especially the lymphocytes; as not staining by dahlia, and hence differed from γ granules; and occurring especially in the cells of bone-marrow. Ehrlich, while he has not publicly repudiated these, leads one to suppose that he now considers them to be not true granules, but nodes of the reticulum of the protoplasm.

Neutrophile Granules (ε).—While a somewhat similar granulation occurs in some animals, cattle, swine, and sheep (Hirschfeld), neutrophile granules of this size, arrangement, and color are found only in man, and hence are considered by Ehrlich specific for him. They are extremely fine, dust-like, and occur in the mononuclear cells of the bone-marrow, a few in transitional cells, and fill the ordinary finely granular cells of the blood. With the Ehrlich triple stain they take a lilac color, and this is really the only specific stain for them, but they also will take an acid stain; hence others name them the "fine oxyphilic granulation," in contradistinction to the eosinophilic or "coarsely oxyphilic."

Neusser's Perinuclear Granulation.—In certain leucocytes, mononuclears especially, and polymorphonuclears, but in some specimens stained with Ehrlich triple stain in any of the leucocytes, are sometimes seen blackish-green granules, which always appear attached to the nucleus. Their size varies much, and they have often a glistening or refractile appearance. Neusser considered them as characteristic of the uric acid diathesis. It has been shown since then [40] that these are in reality artefacts which can be produced by variations in

[40] Futcher, Centralbl. f. innere Med., 1899.

heating and in the stain, and, indeed, with some mixtures may be produced at will, and which bear no relation to the output of alloxuric bodies in the urine.

The Granulation of Lymphocytes.—In well-spread specimens stained by the various modifications of the Romanowski stain are seen fine violet granules in about one-third of the lymphocytes, those with a fairly wide protoplasmic margin. They are not always spherical; their size is between the α and ε; few or many may be present in one cell, yet, as a rule, they are not too numerous to count. They occur also in the large mononuclears and transitionals. They cannot be stained by the Ehrlich stain. By this discovery Michaelis and Wolff [41] consider that they have broken down the sharp line of demarcation drawn by Ehrlich between the granular and the non-granular cells. They are not found in cells from smears of lymph-glands, or of marrow. Ehrlich replied that while it cannot be denied that these were granules, yet they cannot be considered as forming a definite granulation in the sense in which he used the term, since they varied so in number in the type of cells containing them; nor did they occur in all the cells of the class in which some were found; and they required a very special method of staining to demonstrate them.

Fatty granules occur in the leucocytes in cases of hyperpyrexia, are easily recognized and easily stained with Sudan III.

Ehrlich's classification of granules is exceedingly satisfactory as a text-book description, but one working much with the blood finds that nature has not drawn the lines so sharply. Ehrlich admits that both α and ε granules develop from basophile granules. In eosinophile cells there may be α and β granules; apart from this possibility, all α granules are not of the same size, but some larger ones are mixed in, especially in the cells of bone-marrow, sometimes a few in one eosinophile, sometimes many. These may resemble myelin or other degenerations or cell inclusions, and, indeed, the larger or most may be, but excluding these, even in well-stained specimens the size is not always uniform. The view is to be mentioned that all eosinophile granules are the results of phagocytosis of fragments of red cells, or of platelets. Of the γ granules, at least two varieties exist and perhaps three. Cells with various sizes of ε granules occur, and artists employed to illustrate articles on blood absolutely refuse to picture them all of the same size. Again, the line between the α and the ε granules is not always sharp. It may be an individual peculiarity, but in the blood of some patients occurs a large group of cells with acidophilic granules, which one hesitates to classify as neutrophile cells, since they are so large, or as eosinophiles, than which granules they are slightly smaller. This is true in fresh preparations as well as in the stained specimens, and has been admitted by several observers, especially in cases of trichinosis, hence some consider them transitionals (Brown, McCrae, Anderson). And, lastly, especially in smears of the bone-marrow and leukæmic blood, the line between the granular and non-granular cells is exceedingly difficult to draw, since there are so many mononuclear cells with a protoplasm suggesting a faint granulation.

[41] Virch. Arch., Bd. 167, p. 151.

We do not overlook the fact that much depends on technic. Of a set of smears made from the same patient at the same time, but heated and stained with slightly different technic, the granules will appear of somewhat different size and of markedly different color-tone. It is with due allowance made for this that we make the above statements.

Leucocytes.—We give, first, *Ehrlich's classification*, since that is the one in common use.

LYMPHOCYTES (Plate I., 1, also 3 and 4).—These cells are smaller (5 to 8 microns in diameter) or larger (8 to 10 microns) than the red blood-cells. The nuclei are relatively large, round, quite deeply stained, centrally placed as a rule, and have one or two nucleoli. They may be deeply notched, especially the smaller ones, and even suggest a polymorphonuclear cell, but are never just like it. Often there is a clear band between nucleus and protoplasm. The protoplasm forms a narrow rim around the nucleus, is sometimes acidophilic (older cells?), but generally basophilic, often more so than the nucleus, and takes a grayish-green stain with the triple stain. Of other cells the nucleus seems naked. The larger cells of this group have an irregularly staining nucleus with a chromatin network and a faintly granular margin of protoplasm. These latter forms may be exceedingly large in lymphatic leukæmia and in the blood of normal infants. It is rare to see them in other conditions. These cells, if stained with the polychrome methylene blue-eosin stains, show a distinct granulation in about one-half their number.

The lymphocytes constitute from 22 to 25 per cent. of the leucocytes in the normal adult blood, and from 40 to 60 per cent. in the infant's.

LARGE MONONUCLEARS.—By "mononuclear" is meant that the nucleus is round or lobulated but not polymorphous. These cells are two or three times as large as red blood-cells, have a large, oval, vesicular, eccentrically placed, faintly staining nucleus, which indeed may be overlooked, and abundant weakly basophilic protoplasm without granules (Ehrlich stain). Nodal thickenings are present, and by Nocht stain some show a granulation. These cells constitute about 1 per cent. of the leucocytes of the normal adult blood. While in normal blood these cells are practically all large, in other conditions, especially typhoid fever and malaria, this group may be represented by all sizes from that of lymphocytes to large giant-cells. The small forms it is easy to distinguish from lymphocytes, but they occur very seldom in normal blood. (Plate II. The group 9–20 contains many.)

TRANSITIONAL CELLS (Plate I., 5).—These cells resemble the large mononuclears, but are often larger,—in fact, the largest cell of the blood. The nucleus is much notched, giving the so-called "wallet" or "saddle-bag" nucleus. The protoplasm stains quite deeply in Ehr-

lich's stain, and often presents a very few neutrophile granules. These constitute from 1 to 3 per cent. of the leucocytes of the normal adult.

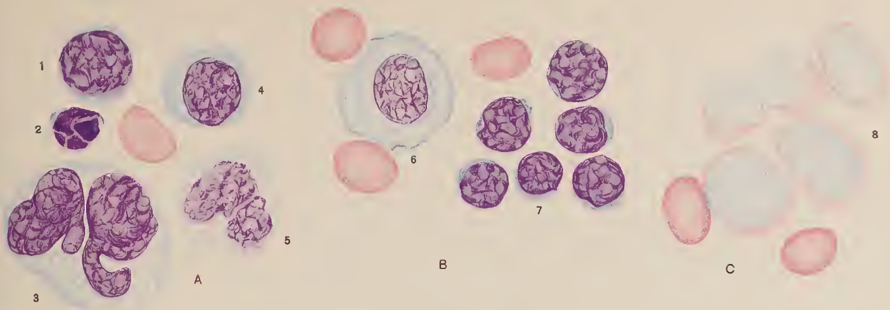
Polymorphonuclear Neutrophiles (Plate I., 6, 7).—These cells, which constitute from 70 to 72 per cent. of the leucocytes of the adult and from 18 to 40 per cent. of the child's, are somewhat smaller than the transitional cells. When spherical they are about 10 microns in diameter, but in a well-spread smear, in which they have flattened out upon the glass, they may seem about twice this size. The amount which they have flattened explains their varying size, so often deceptive in smears of unequal thickness. The nucleus is characterized by its polymorphous nature and its deep stain, due in many cells to pycnosis. It may be a strand variously bent, or small fragments, two or more in number, connected by fine filaments. The protoplasm takes a faint acid stain. It is well filled with the neutrophile granules which may cover the nucleus. When migrated these are the ordinary pus-cells. They contain glycogen in certain conditions.

Eosinophiles (Plate I., 2).—These cells are of the same size or perhaps a little larger than the preceding. The nuclei may have the same shape, yet less pycnotic, fainter staining ones are the rule. The protoplasm is often slightly more abundant, and is filled with eosinophilic granules, which do not often lie upon the nucleus. These cells constitute from 2 to 4 per cent. of the normal leucocyte count.

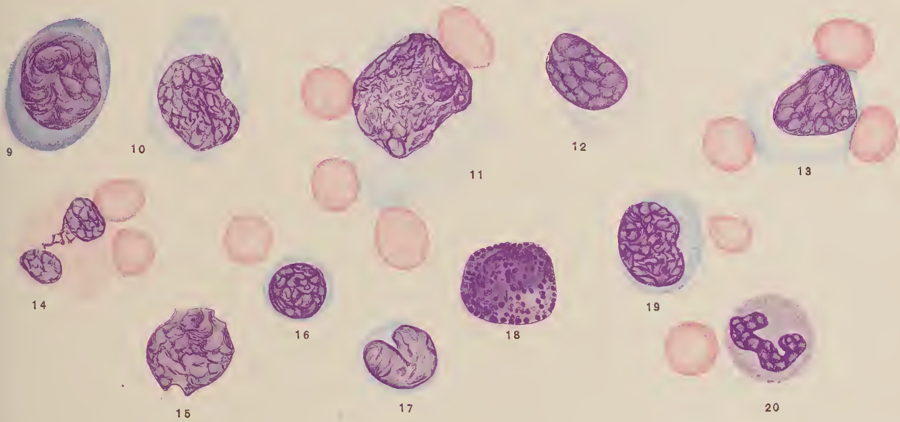
Mastzellen (Plate I., 8).—This name is given, perhaps incorrectly, to any cell with basophilic granules. It is not at all certain that these are in any way related to true Mastzellen of the connective tissues. These cells are about the same size as the preceding, but more often smaller. The nucleus is polymorphous, very faintly staining, often trilobed. The protoplasm contains a variable number of granules of different sizes, yet for the most part as large as α granules, which form a band around the nucleus. These granules are not stained by the triple stain, hence one sees only the spaces occupied by them. (These are probably the reticulated or vacuolated cells of Uskow.) They stain best in thionin and are said to take a metachromatic tone. These cells constitute about 0.5 per cent. of the total count. They have every appearance of old cells: are small, do not spread well, but look shrivelled, with acidophilic protoplasm and polymorphous nucleus.

The leucocytes found in pathological conditions are:

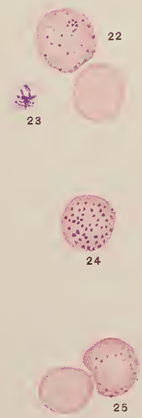
Myelocytes (Plate I., 9, 11, 14, 17).—While any cell of bone-marrow is, strictly speaking, a myelocyte, by this term is generally meant one with a round nucleus and granular protoplasm. Neutrophilic and eosinophilic myelocytes occur. Their size varies from that of the large mononuclears to that of red corpuscles. The largest and smallest neutrophile myelocytes are found in the blood only in



LYMPHATIC LEUKÆMIA.



LEUCOCYTES OF NORMAL BLOOD AND MALARIA.



TRYPANOSOMA GAMBIENSE,
FROM A CASE OF "SLEEPING SICKNESS."
STAINED WITH HASTING'S MODIFICATION OF
ROMANOWSKI'S STAIN. ALL DRAWN TO SAME SCALE.

BASOPHILE GRANULES
OF RED BLOOD CELLS.

F. S. Lockwood.

myelogenous leukæmia, but a few the size of the granular leucocytes in any condition with a considerable leucocytosis. The characteristic point is the shape of the nucleus, which is either perfectly round, oval, indented or kidney-shaped, but never polymorphous or pycnotic; if it were, the cell would count as an ordinary leucocyte. It is usually centrally placed. It is impossible to draw a line between a myelocyte and polymorphonuclear (Plate I., 12, 13), since every possible gradation occurs between them, but one soon forms a standard for himself. As myelocytes are counted all with round, oval, or kidney-shaped nuclei. In the latter case the nucleus should occupy at least one-half of the cell, with its convex edge forming a part of the periphery and the indentation at about the centre of the cell. It is not indented on both sides. Such a nucleus will not be stained diffusely by any good nuclear dye. A cell with nucleus more compact, distorted, or diffusely stained than this ranks as a leucocyte. The specification "round or oval nucleus" needs a further qualification, for one sometimes in leucocytes sees such nuclei, but they are relatively small (occupying only about a quarter of the diameter of the cell) and stain diffusely. One believes that could he get a side view of them, they would be polymorphous; also their lack of chromatin net-work is enough to prove them leucocytes. For the question of the motility of these cells, see Wolff.[42]

Some are full of granules, some have but few, and they scattered. The very large ones occur in the bone-marrow and in well-made specimens of the blood of myelogenous leukæmia, but the most are broken up, and only a large faint nucleus and granules free in the plasma are seen. Some have a large nucleus and narrow rim of protoplasm, some a smaller nucleus and more protoplasm, and some are very small.

Eosinophile Myelocytes (Plate I., 14).—These are the exact analogue of the preceding, and occur in much the same conditions, but less often and in much smaller numbers. They are found especially in splenomyelogenous leukæmia and in anæmia pseudolymphatica infantum.

Small Neutrophiles; Pseudolymphocytes.—These cells have a round, intensely staining nucleus and a narrow margin of protoplasm full of neutrophile granules. Their size is about that of a lymphocyte. They are rare, occurring especially in pleuritic exudates, and are supposed to arise from fragmentation of the polymorphonuclear cells.

Irritation Forms.—The description given of these cells is the following: They vary in size from a lymphocyte to a large mononuclear, but resemble the former more; the nucleus is round, of a bluish-green color (Ehrlich stain), often eccentric and without a chromatin net-work; the protoplasm stains an intense rich brown; these have no granules. Türk considers that they have the same occurrence and meaning as myelocytes.

[42] Deut. med. Wochenschr., March 5, 1903.

Uskow's Classification.—This writer has given a classification of leucocytes based on specimens stained with the triple stain, more objective than that of Ehrlich, and although not in common use, yet valuable to bear in mind when using any classification.

A. *Lymphocytes.*—Cells with a round nucleus, narrow ring-like rim of protoplasm separated from the nucleus by a clear sharp circle, the nucleus and protoplasm staining intensely.

(1) *Small Lymphocytes.*—The size of red blood-cells or smaller, with a uniform ring of protoplasm.

(2) *Lymphocytes.*—A little larger than red blood-cells, the rim of protoplasm often thicker on one side, giving the seal-ring appearance, or with two or three prominences.

B. *Transparent Corpuscles.*—Characterized by the quantity of protoplasm which with the triple stain remains colorless. The nucleus is homogeneous, round, oval, or bean-shaped, usually eccentric and stains a feeble pinkish shade.

(3) *Small transparents,* size of large lymphocytes, or a little larger, often in the form of a square with rounded corners.

(4) *Large,* three to five times that of a red cell; the nucleus eccentric.

(5) *Lobulated Forms.*—These are the largest cells of the blood. The nucleus is on one side, usually toward the centre of the corpuscle, with one or two deep indentations.

C. *Transitional Cells.*—These have many of the characteristics of lymphocytes and transparents. They are rich in a protoplasm which is sometimes slightly granular and always takes a good stain, but less intensely than that of lymphocytes. The nucleus stains more deeply than the protoplasm and usually has no clear ring around it.

(6) *Small Transitionals.*—Resemble giant lymphocytes or colored small transparents.

(7) *Transitionals,* are larger.

(8) *Lobulated forms,* the largest of this group.

D. *Polymorphonuclears.*—The nucleus intensely staining and of various shapes. Protoplasm abundant, coarsely or finely granular.

(9) With thick, rod-like nucleus, which stains rather feebly. Granules smaller than those of the other neutrophiles.

(10) Nucleus like a bent rod, often twisted at one end.

(11) Multinuclear, the fragments, however, connected by strands of chromatin.

Uskow considered the above classification based on age. As young forms, he considered the small and large lymphocytes and the small transparents; the ripe elements were all the transitionals and the large and the lobulated transparents, while the over-ripe forms have polymorphous nuclei. The classification is based on Ehrlich's stain, and hence cannot, as a whole, be as well applied to specimens otherwise stained. It is, however objective, and gives a much better description of the cells actually found than does the Ehrlich.

Differential Counting.—For a differential count a satisfactory classification is a necessity. Since we know so little of the relationship between the various leucocytes, of their age, their changes, their origin, and of their function, the only classification possible would be a purely morphological one. Pappenheim has proposed such a one, but his is too complicated for clinical use, hence Ehrlich's, being the simplest, still obtains. Ehrlich separates in the normal blood, small mononuclears, large mononuclears, transitionals, polymorphonuclear neutrophiles, eosinophiles, and Mastzellen.

By small mononuclear is meant any non-granular cell smaller than a polymorphonuclear neutrophile. This group would include, there-

fore, all lymphocytes and the small transitionals and transparents of Uskow. As large mononuclears are classified any non-granular cells larger than a polymorphonuclear neutrophile, with a round or oval nucleus; any cell within the same size limits, but with an indented nucleus, is called a transitional. The polymorphonuclears, both neutrophiles and eosinophiles, are clear enough. As a Mastzell, is counted any polymorphonuclear cell without granules (Ehrlich's stain), or with blue granules if methylene blue is used.

For normal blood this classification is satisfactory, but for pathological conditions many objections arise. While the lymphocytes seem to form one distinct class (Plate I., 3, 4, 15, 20), the other mononuclear cells it is impossible to classify on the staining character of their protoplasm, but this point is quite certain, the group of large mononuclears has large, medium, and small forms, and any line dividing this group is purely arbitrary; it increases the number of the lymphocytes by cells which do not belong there, and diminishes a group which should not be divided. This is best seen in typhoid fever and malaria, in which diseases the group of the transparent cells of Uskow is increased as a whole, both the large and small forms; and in the so-called lymphatic leukæmia, in many cases of which the mononuclear cells are certainly not lymphocytes.

Neither is it just to separate groups on the basis of an indentation of the nucleus. Ehrlich's name "transitional" still exists, but as soon as, by means of the triple stain, he discovered the myelocyte, he saw at once that the transitionals were no longer necessarily a step in the development of a polymorphonuclear cell. It is now generally agreed that these large cells with indented nuclei are only older (senile) forms of those with oval nuclei, and need no longer be counted as separate. As regards the transparents and transitionals of Uskow, it may well be, as some suggest, that the younger cells have a more basophilic protoplasm, while the older cells have an acidophilic; or these cells may be of really distinct origin.

The line between myelocyte and leucocyte is very hard to draw, since no line can exist in an unbroken series of intermediate forms.

In leukæmia to draw a line between large mononuclears (Plate I, 16, 19, 21) with deep staining protoplasm and granular myelocytes is also very hard, since perhaps here also no line exists; yet in well stained specimens one is in doubt concerning but few cells.

One group of cells does confuse us, especially in heated specimens, cells which are represented merely by a faintly staining nucleus, and that this is a nucleus one is often in doubt; especially if one control his count by one on the fresh blood, then he is sure all are not leucocytes. Such cells should not be passed over, but counted as "undetermined cells," for only in that way will the percentage of those groups which are more resistant be fairly correct. These undetermined cells are almost all large mononuclears, yet if many are found on preliminary examination of a smear, that smear should not be used for a differential count.

To differentiate eosinophiles and neutrophiles there should be little difficulty in a well-stained specimen, and yet in certain cases the question is very hard, and one doubts for that case the specificity of granules. We believe that most observers do not pay much attention to a point formerly emphasized, the characteristic lilac tint of the neutrophile granules; that they do not believe that true eosinophile granules may be as fine as neutrophiles and distinguished by their color-tone alone, as do some who have written concerning eosinophilia. To most of us neutrophile granules are fine and eosinophile coarse, and little attention is paid to their color except as a basis to criticise the staining mixture used. One further point is important. It is customary to count neutrophile myelocytes in a class by themselves, but eosinophile myelocytes with the eosinophile leucocytes. We are not at all sure this is a fair method.

For our differential counts we use specimens stained with Ehrlich's triple stain, and separate first the granular and non-granular cells. Of the latter the lymphocytes might be counted alone, and all other mononuclear non-granulars in one group, unless they be increased, when one could separate transparents and transitionals (Uskow) on the basis of the protoplasmic stain, or large mononuclears and transitionals (Ehrlich) on the basis of the shape of the nucleus. Yet in routine ward work and for the sake of uniformity we still separate small and large mononuclears, using the polymorphonuclear neutrophile as the size-line, but count large mononuclears and transitionals together. The granular cells are divided as neutrophiles, eosinophiles, and basophiles. Separate classes are made for ε myelocytes and α myelocytes. Nucleated reds are also counted and calculated as "number per thousand leucocytes."

The list of cells is, therefore, the following, using the customary abbreviations: S. monos., or s.m.; l. monos., or l.m.; tr.; pmn. ϵ, or pmn. n.; pmn. α, or pmn. eos.; Mastz., myeloc. ϵ, myeloc. α, nucleated reds, normobl., intermed., megalobl.

For a differential count a mechanical stage should be used, and at least five hundred, better one thousand, leucocytes counted. Yet one can get a fair idea of a slide without a mechanical stage. Some keep count with a pencil on a paper ruled into columns for each group; others use a slide-box divided into compartments by slides, into which he drops beans, one bean for a cell. Since one starts always with five hundred or a thousand beans, the mathematics of this calculation are easy.

Many use specimens stained with the various polychrome methylene blue-eosin stains for differential counting, with confidence of their ability to distinguish the various granulated cells, and the added advantage of better-stained nuclei and stained basophilic granules. In ordinary use this is very well (Plates II., III.). The average Ehrlich triple stain mixture in use is a poor fluid, and gives a poorer picture than these. Ehrlich's neutrophilic granulation has not gained quite the clinical importance which he expected, but it is only fair by Ehrlich to use the term "finely granular cells" or "fine acidophilic," in case other stains are used, and reserve the term neutrophilic in connection with his triple stain, for although the color-tone of the two granulations is different when the former stains are used, the result is not so specifically neutrophile, as with the triple stain.

With these stains (Nocht and its modifications) all nuclei stain much better than with the Ehrlich. The protoplasm also stains much better, an intense blue with very beautiful net-work, or a diffuse blue, or a red. The finely granular cells present a diffuse haze of purplish granules, and many of them can be made out easily and

clearly and their tint seen, but the picture is not so beautiful as in an Ehrlich specimen. The eosinophile granules take the eosin. For very careful work it is advisable to count two specimens, one stained with Ehrlich's triple stain for granules, one with hæmatoxylin-eosin for nuclei; by comparison these will correct each other.

We hope that soon much more differential counting will be done with fresh blood preparations. It is rather hard and often inconvenient, but perhaps more accurate than with stained smears, since what one loses in the tint he gains by avoiding artefacts and broken-down cells.

Bone-Marrow.—The study of the bone-marrow is a subject of primary importance. In it are found practically every cell which occurs in the blood in almost every condition; that is, a large number of those cells which are unusual in the peripheral blood, and a complete series of transitional forms between different groups; hence this study renders the blood-pictures more intelligible.

The study of fresh marrow is especially important; that of the ribs of young babies, especially of those born prematurely, is best. Fragments removed in operations for empyema are excellent, and autopsy specimens if fresh enough. It is surprising how quickly some of the interesting mononuclear forms, "young" cells, disintegrate. The large form of myelocytes also soon disappear, and in leukæmia the marrow may soon be of very little value, showing only a confused mass of nuclei in a cloud of free granules. A small piece is squeezed in a pair of forceps and a small drop of the exuding marrow picked up on a cover-glass and at once pressed down onto a slide. Spicules of bone must be avoided. Very rapid work is necessary, since the drop dries very rapidly. The marrow may be diluted with salt solution if desired. For stained specimens, the stroke method is the most useful; that is, the marrow is smeared in lines on the cover-glass by drawing this across the end of the bone. The specimen is allowed to dry in the air and then may be fixed and stained just as blood smears. If the marrow is fatty the smears do not turn out well. If, fixed by heat, it is well to remember that it is easier to underheat than it is to overheat, and the easiest method is to place the cover-glass on the copper plate, smear up, at the spheroidal point (that is, the point at which the drop of water does not boil but merely rolls off the plate) for forty-five or more seconds. Such specimens will for the most part have good areas for study, especially at the edges of the thick portions, and a few such fields are all that is desired. Specimens made thin, in the hope that the surface will be uniformly good, are usually failures, for those too thick are better than those too thin if heat and the Ehrlich stain be used.

Bone-marrow varies much. In some places will be found nests

of nucleated reds in enormous numbers; in other places nests of leucocytes, myelocytes, and of intermediate forms. Different parts of the same rib vary, as we have found to be markedly the case in infant marrow. Since the marrow in different bones and in different parts of the same bone varies so, it is impossible from a limited search to say what is the general medullary condition of that case (Grawitz), and this may explain the lack of evident relation between a marrow and a blood picture.

While it is impossible to really count the cells of the marrow, yet differential counts can be made of those found in measured areas.

Nucleated Red Blood-Cells.—The term "erythroblast" is used by some to mean a nucleated red blood-cell; by others a colorless ancestor form of these. We use it for any nucleated red cell.

(1) Normoblasts. (*a*) *Howell's Mature Nucleated Reds* (Plate I., 29).—These are the color of the non-nucleated red blood-corpuscles, with a pycnotic nucleus 3 microns or slightly less in diameter, sharply defined, without a chromatin net-work, dense, homogeneous, structureless (triple stain), of a dense uniform blackish-green color, often vacuolated, hence in the nucleus is often a bright spot in the fresh and an unstained area in the stained specimen. These nuclei are so characteristic that they may be recognized even if not surrounded by protoplasm. They often present amitotic figures, giving rise to rosette forms of two to four or even twelve fragments. Sometimes these fragments are all connected by strands of chromatin (see Plate I., 34, Fig. 113, *c*). This nucleus is often surrounded by a clear zone which probably represents space left between the contracting protoplasm and nucleus. The nucleus may not occupy this space, but rest upon a margin of the red cell or even at some distance from it. This is explained by Ehrlich by the weight of the nucleus—when the specimen is made the cells are violently thrown into a new position, which centrifugal force throws the nucleus out of the cell. And yet this will not explain all free nuclei, since they are seen in specimens made in various ways and in sections as well. Pappenheim and Israel claim that in leukæmia especially such free nuclei result from the degeneration of the surrounding protoplasm.

From these cells with very pycnotic nuclei, in which can be seen no structure whatever, are all gradations with the chromatin structure more and more evident till we reach (*b*) *Howell's immature nucleated reds* (Plate I., 30, Fig. 113, *a*). These are of a little larger size than an ordinary red blood-cell with color perhaps a little paler; the nucleus is slightly larger, with the chromatin fibres radially arranged (in leucocytes it forms a meshwork [Pappenheim]), while clearly seen mitotic figures are not rare. Division of these cells is rapid, requiring but fifteen minutes. In the bone-marrow this is the dominant red cell.

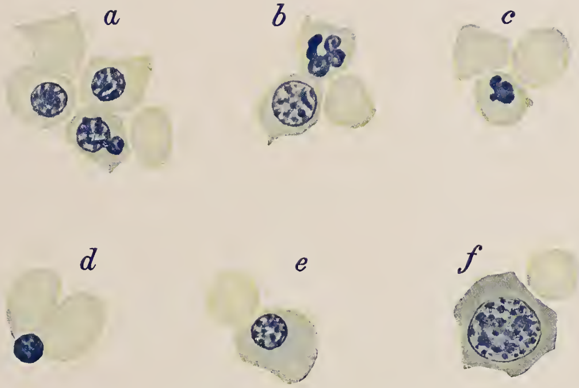


FIG. 113.—Nucleated reds from the blood of a fœtus 15 cm. long. *a*, mature nucleated red; *b*, intermediate form and rosette; *c*, mature red, nucleus fragmented; *d*, free nucleus of a mature red; *e*, mature red, polychromatophilic cell; *f*, polychromatophilic megaloblast.

These two forms are generally called normoblasts, although the larger immature forms are by some classed as intermediates. They are the precursors of the ordinary non-nucleated red blood-cell, but do not reach the circulation of a normal adult except as an anomaly. Their appearance in the general circulation indicates an increased activity of the bone-marrow, such as occurs after hemorrhage. They appear in the blood of a child more readily than in that of an adult. Many occur in pernicious anæmia, more in splenomyelogenous leukæmia, and some in post-hemorrhagic anæmias. As in the marrow so in the blood these cells occur in groups often, and lie free from the rouleaux (since heavier than the others?).

These cells are "orthochromatic" normally; that is, they stain like the ordinary non-nucleated reds (oxyphilic). There is a group of fuchsinophilic cells which some consider young forms, Engle a distinct group; other cells, especially in pernicious anæmia, are polychromatophilic.

"Blood crises" is the term given by v. Noorden to the periods, usually in the convalescence of an anæmia, during which for a few days enormous numbers of nucleated reds and a leucocytosis will be found, and this followed by a jump in the red blood-count. The nucleated reds then disappear and the increase in the count is less rapid until perhaps another crisis occurs. V. Noorden reported cases with gains of half a million cells in four days. Since a leucocytosis is also present, he considered it a transitory increase of activity on the part of the bone-marrow to regenerate the blood. Normoblasts are, however, not the only red cells increased, and a crisis is not always a sign of improvement (see page 534). It is to be distinctly emphasized that the number of nucleated reds in the circulating blood is a poor index of the activity of the bone-marrow, much less so is the actual count of red cells. The bone-marrow may be able to maintain the count at the normal level through the most strenuous efforts.

Intermediate Red Blood-Cells (Plate I., 31).—This is an exceedingly indefinite term; by it are meant cells which are not quite large enough to be called megaloblasts, and yet are larger than normoblasts; large cells with the nucleus of an immature red, or smaller cells of normoblastic size with a relatively large reticular nucleus. If one systematically measures all nuceated reds in a specimen, the small number of these cells is striking unless one include the immature nucleated reds of Howell (Fig. 113, *b*).

Megaloblasts.—In the marrow always are found nucleated reds (Fig. 113, *f*), which are from two to four times the size of an ordinary red blood-cell. They are round or oval. The protoplasm makes up most of the cell, and is often polychromatophilic, but the polychromatophilia may be explained rather by youth than by degeneration;

in the case of the primary anæmias they are rich in hæmoglobin; in the normal marrow they are often pale. In stained specimens the nucleus is large, plump, round or oval, especially the former, usually central; in fresh smears it is easily seen, and has a good chromatin net-work if a good nuclear stain be used. It is thus seen that these cells are larger as a whole, and have a larger nucleus than have other nucleated reds, but what should they be called?

In all reports of blood cases it is necessary to know the writer's definition of a megaloblast, for opinions vary much. Some demand a large cell, some a large nucleus. Our rule is that both of these specifications must be fulfilled, and for reasons to be given later we ask that the size of the nucleus shall be at least that of an ordinary non-nucleated red (7.5 microns). Pappenheim and others consider that megaloblasts have no direct relation to normoblasts, and that from certain fine points concerning their nuclei a megaloblast may be recognized, even though it be as small as a normoblast.

It is true that the megaloblast of pernicious anæmia does differ from the large nucleated red of bone-marrow. The size of both is about the same, but the normal bone-marrow megaloblast has usually a round nucleus with very distinct margin and chromatin net-work. That of the cell of the blood in pernicious anæmia is more often oval, much less distinct in the fresh blood and stains much fainter in the smear, has a less definite nuclear membrane and chromatin structure; the cell looks flabbier; but these differences are slight and inconstant.

The significance of the appearance of megaloblasts in the blood has been the subject of dispute; Ehrlich, considering that they never occur in the normal bone-marrow of an adult, believes them to be the product of a megaloblastic degeneration of this tissue due to a toxine, a reversion to the embryonic condition, and others say "to the amphibian," and that any attempts to break down the distinction between normoblasts and megaloblasts fail from the fact that in pernicious anæmia the blood is megaloblastic. (We are inclined to think that the expression "reversion to the amphibian type of blood" is much too often used. The only amphibian we have studied whose blood resembles that of pernicious anæmia is the batrachoseps, and one would hardly call an hæmoglobinæmia a "reversion," although that is the blood condition in some worms, hence could as easily be called a "harking back.") Such megaloblastic degeneration may explain their large numbers in the marrow of pernicious anæmia and some other conditions, but we fail to find any recent observer who has not easily found them in all normal marrows. In our study of bone-marrow, in which many nucleated reds have been measured, we have found the predominant cell the immature normoblast, with a nucleus between 3 and 4 microns in diameter. The next most common cell is a megaloblast, with a nucleus of 7 microns or over in diameter, or in one axis if oval. Between these large cells and the immature normoblasts occur every intermediate size, and yet the group of all of these is not as great as that of the large cells which constitute about 15 per cent. of the total number of nucleated reds. This applies to specimens with nucleated reds in moderate numbers, not those with innumerable nucleated reds in nests.

It has been shown that in certain animals the nucleated reds are grouped in islands, with the centre of megaloblasts surrounded by zones of cells of diminishing size to the periphery, where normoblasts are found (Bunting). The develop-

ment is not from centre to periphery alone, for in each zone, and especially the peripheral, signs of active regeneration are found, and each cell can produce its like as well as a smaller form. Ordinarily it is the periphery of these islands alone which furnishes new cells to the circulation, but if because of an over-demand the islands be encroached upon, the larger cells of the interior will be thrown into the circulation. Bunting has shown in a most interesting manner that by various blood poisons the peripheral zone of these islands may practically be stripped off. Should this occur in pernicious anæmia it is easy to understand the large numbers of megaloblasts in the blood, yet why nucleated cells reach the circulation in some cases and not in others is not clear, since in some cases the marrow is very rich in megaloblasts and none in the peripheral blood, and again the blood will show great numbers (blood crises). The bothriocephalus anæmia shows that certain specific toxines can produce this " megaloblastic degeneration" of the bone-marrow, for as soon as the worm has died the blood at once begins to return to its normal condition.

Karyokinesis of these large cells occurs in the peripheral blood, especially in severe anæmia, and is usually one of the final phenomena.

MICROBLASTS.—By microblast is meant a very small nucleated red, under 6 microns in diameter, with a small pycnotic nucleus. These occur in the circulation in severe traumatic anæmias, never normally; some appear perfect cells, and others as if pinched off from larger cells. The former may be the forerunners of microcytes.

The *fate of the nucleus* of the red has been the subject of great discussion, and still is. Two views have been held: (1) that the mature normoblast extrudes its nucleus (Rindfleisch, Howell, *e.g.*), and (2) the intracellular destruction by karyorrhexis and karyolysis (Kolliker, Neumann, *e.g.*). Those who hold this latter opinion admit that the nucleated red may even be seen to extrude its nucleus, but consider that this is pathological; that normally the nucleus goes to pieces in the cell, either by solution or by fragmentation, and the process which predominates varies with the disease. Many writers believe that all of these methods may obtain; Ehrlich, for instance, that the normoblastic nucleus is extruded, and that the macroblastic is absorbed; Pappenheim, that for both it is intracellular; Bloch, that either is possible. Whether or not the nucleus is extruded or disappears within the cell, the cell then flattens somewhat, becoming more disk-shaped and then biconcave. But not all are biconcave, some are spherical, especially in the embryo, a point emphasized by those holding the absorption view. The " degenerations" or nuclear fragments described by Vaughan (page 401) and by Cabot (page 446) indicate the intracellular type. Just at present the extrusion idea seems to obtain, but perhaps chiefly since so many need the nuclei to explain the origin of the platelets. The free nuclei which some emphasize in stained specimens may have been thrown out of the normoblast by the centrifugalized force of the sudden motion of the cells as the specimen is made, and the nucleus of the megaloblast remains in the cell, since its specific gravity is nearer that of protoplasm (Ehrlich, Pappenheim).

The *changes in the nucleus* are important. By a " pycnotic" nucleus is meant one diminished in size, dense, homogeneous, sharply defined, sometimes vacuolated, without any good chromatin net-work. The Ehrlich stained cells show no structure at all, only a deep uniform chromatin stain, but good nuclear dyes show traces. There seems a decrease of nuclear fluid and a solution of the chromatin in it. This is a preliminary step of karyolysis or absorption, the nucleus taking

a fainter stain till it cannot be distinguished from the surrounding protoplasm. It may precede karyorrhexis or fragmentation of the nucleus, which fragments may then disappear by karyolysis. The normoblastic nucleus may by amitosis divide into polymorphous forms, with two or even twelve fragments (Plate I, 34) of equal or unequal size, and usually united by a filament, giving beautiful rosette pictures. Some attribute this to karyorrhexis rather than to abortive mitosis. In a recent case during a blood crisis 55 per cent. of the erythroblasts were of this description, some having the nucleus in even twelve lobes. Another method suggested by some specimens is the disappearance of the most of the nucleus, leaving a few chromatin strands and masses. The study of nuclear degenerations should never be made with specimens stained with the Ehrlich stain. That is least adapted for nuclear changes. One other point well seen in the bone-marrow is the varying hæmoglobin tinge of the corpuscles, these cells showing a much wider variation than those of the blood.

This could be explained on the ground that the development of hæmoglobin was an intracellular matter, a gradual process. Some cells seem to reach complete hæmoglobin development after they lose their nucleus, some before. The question is of interest in the study of anæmias. Are these pale cells in the circulation of fixed composition, or only immature and later develop more hæmoglobin? Can a normal cell lose some hæmoglobin; that is, can the color-index rise and fall, and yet only the same cells remain? When the color-index falls is it because the cells are like depreciated currency, and it rises when these are recalled and better issued in their place? The question may have little practical importance, and yet if discussed the much desired result should be greater care in the use of terms with which we describe our cases. Arguments from comparative anatomy are not satisfactory, yet the evidence goes to show that in lower vertebrates the red cells can complete their development in the circulation, while in the mammals an imperfect cell is said to be incapable of further development. Among others, Gaule and his pupils believe in a hæmoglobin "store" in the body, which in case of need is carried into the circulation in new corpuscles and returned when the extra cells are withdrawn.

We fear the great trouble is in the hæmoglobinometers, and that until careful work is done with the best instruments the question will remain unsettled.

Origin of Red Blood-Cells.—That the ordinary non-nucleated red blood-cells come from the nucleated reds is now doubted by few. Up to the end of the fourth week of embryonic life all of the blood-cells are nucleated. From that time on the number of the non-nucleated cells increases until at the third month only about one-sixth to one-eighth are nucleated. At the fifth month they are still numerous, but at birth it is rare to find any nucleated red cells in the blood.

In the earliest embryonic life the vessels are formed from solid cords of cells, the peripheral ones of which become the endothelial lining of the vessel wall, the internal cells the corpuscles. This process may occur in almost any part of the developing organism, and perhaps also in the adult when there is new formation of blood-vessels. In addition, in the embryo many mitoses are found in the nucleated reds of the circulating blood.

Before the third month the liver has become the chief seat of blood formation; after the fifth month the spleen and the lymph-glands take up the task; and at last the marrow becomes the chief organ. In the child the marrow of the whole skeleton has this function, but at about puberty and during adult life only the ribs and some of the flat bones. Howell considers that callus, for instance that fol-

lowing a fracture, may in the adult also for a while furnish centres for hæmogenesis. In the adult it would seem as if the spleen could resume this function in leukæmia and anæmia.

Removal of the spleen causes little anæmia, but after about a month begins a long continued rise of small mononuclears, and in some conditions the blood is even leukæmic, and then after about twelve months an eosinophilia of even extreme degree. These phenomena are now explained as manifestations of the vicarious activity, first, of lymph-glands then of the bone-marrow, for the spleen. The chief function of the spleen seems to be to remove old reds and leucocytes from the circulation, and the acute spleen tumor in some conditions is due to the great number of leucocytes ingested (spodogenic splenic tumor).

In the embryo the blood-cells are at first without hæmoglobin. At this time there are no true leucocytes and none appear until after the formation of red cells is active. The embryologists have shown that before the appearance of leucocytes in disease of the embryo the red blood-cells are amœboid, perhaps phagocytic, which is interesting, since in certain blood diseases of the adult there is at least a suggestion of these two functions.

In lymphatic leukæmia (certain acute cases) the view is still held by some that the increased cells are "red cells" without hæmoglobin, and the converse Pappenheim holds to be true in severe anæmias, some of the large lymphocytes developing to megaloblasts instead of to small lymphocytes.

Howell was the first to demonstrate in the cat "ancestral corpuscles" which resemble the red blood-cells of reptiles, being large, oval, semifluid red cells, with a deeply stained oval nucleus. These cells were later described by Engel as "*metrocytes of the second generation*," those of the *first generation* having a large chromatin-rich nucleus. Later such cells never, normally at least, reach the circulation, and Engel thinks they are no longer formed.

For the study of the young red cells the blood of embryo mice is to be especially recommended. Here a great variety of changes in the nucleus and in granulation of the protoplasm may be demonstrated.

White Blood-Cells.—For the best recent study of bone-marrow, see Schur and Löwy.[43]

A. GRANULAR CELLS

I. Neutrophiles. Neutrophile myelocytes.

1. Typical myelocytes; cells from 12 to 15 microns in diameter, with a large round nucleus, the protoplasm scanty, forming often a thin rim around the nucleus, and finely granular. These cells are by far the most numerous in the marrow, but on account of their size seem even more so than is the case. The nucleus is often hard to make out. In the bone-marrow may also be seen beautiful larger myelocytes with faint nuclei, which, however, are not often found well preserved since they go to pieces so readily, and which occur in the blood only in leukæmia, "Cornil's marrow cell." In the fresh marrow some of the myelocytes have a very small, dense, round nucleus, which we think is a post-mortem change from the loss of nuclear fluid. All transitions from those cells with a round nucleus to the typical leucocytes are present on the one side, all transitions from large mononuclears with but few or no granules to those full of granules, on the other.

[43] Zeitschr. f. klin. Med., Bd. 40.

2. Cells similar to the above, but much smaller. The nucleus indented, or slightly polymorphonuclear, but staining faintly,—" transitional cells."

3. Typical leucocytes.

II. Eosinophiles, always relatively few in number.

1. Eosinophile myelocytes. Large cells with pale nucleus, scanty protoplasm filled with eosinophile granules, otherwise similar to the above mentioned neutrophile cells.

2. Similar to the above, but smaller; the nucleus indented or slightly polymorphous. All gradations may now be found to typical

3. Eosinophile leucocytes.

III. Basophiles.

1. Mastzellen, which, however, are rather rare to find. These cells have nuclei of a variety of shapes, yet usually polymorphous (see page 472). Mononuclear Mastzellen occur (Engel). These are, at least, hard to recognize, since the young α and ε granules are quite basophilic.

2. Various cells with violet granules, which vary in size. The cells are rather small. These, although containing basophile granules, are not typical Mastzellen. In this connection it should be mentioned here that the β granules may occur in the eosinophile cells or by themselves.

3. Polymorphous cells, with fine basophile granules. These may be neutrophile cells stained by the "tricky" methylene blue (Schur and Löwy).

B. NON-GRANULAR CELLS

1. Lymphocytes. Size of red blood-cells, nucleus rich in chromatin, protoplasm a narrow rim. These cells are the second most numerous cells and often seem naked nuclei.

Among these are the "protoleucocytes" of Osler, solid-looking lymphoid elements from 2.5 to 5 microns in diameter, which resemble free nuclei; some have a rim of protoplasm. From these "erythroblasts" develop (see page 485).

2. Medium-sized lymphocytes with more protoplasm and a smaller often eccentric nucleus.

3. Very large cells with general character of lymphocytes which occur in the blood in some acute leukæmias, but none normally: "Large lymphocyte" (Ehrlich, Fränkel, Pappenheim): Grawitz's "unripe cell." Wolff's "indifferent lymphoid cell," Naegeli's "myeloblast," Troje's "marrow-cell.' The nucleus stains faintly, is seldom lobulated, is very pale and poor in chromatin, the protoplasm is faintly basophilic.

Also to be mentioned are cells, common enough, which in the fresh exactly resemble normoblasts (immature), except they have no

hæmoglobin. The nucleus is that of Howell's immature red, perfectly round, with sharp margin, distinct chromatin net-work, and clear hyaline protoplasm. They are from 9 to 12 microns in diameter. These are the erythroblasts of Osler, Löwit, and Howell.

Löwit described as "leucoblasts" cells with relatively large nuclei containing one or two chromatin masses which are sometimes irregular in shape, and from which a system of delicate lines and bands radiate to the nuclear membrane, which membrane is distinctly doubly contoured, and has on its inner surface projections which are connected with the infranuclear net-work. Concerning their method of division, upon which Löwit lays much stress, it is not easy to decide.

Ehrlich believed the true lymphocyte came from the lymph-glands; others say from the bone-marrow. Some have tried to distinguish morphologically those from the marrow from those from glands (Rubenstein). The question now is, "Do any come from lymph-glands?" thus admitting that typical "lymphocytes" are an important constituent cell of the marrow. Michaelis and Wolff tried to differentiate these cells on the basis of their future history, the lymphocytes from lymph glands remaining such, the "lymphoid" cells of the marrow being capable of further development to a granular cell. But this capability does not aid us much in saying which the individual cell now before us is, although these writers did describe slight differences in their staining reactions. And yet this distinction between lymphocytes and lymphoid cells is probably just. Many workers fall back upon such indifferent lymphoid cells as young forms, naming them "protoleucocytes" (Osler), etc., and consider that from them develop colorless cells which correspond to the leucoblast and erythroblast of Löwit and Howell, and from these the whole series of reds and whites. The lymphocyte with diffusely staining notched nucleus (Rieder's cell) is probably an old form of lymphocyte. The small mononuclears with round vesicular nucleus, delicate chromatin net-work, and rather broad band of basophilic protoplasm with smooth margin, are young cells which resemble only in appearance those of the normal blood, and themselves do not belong there. The lymphocytosis of young babies is unquestioned, and yet the most rational explanation for this is that there is an overproduction of leucocytes which did not exist at birth, since then there was no function for the cell. Yet this would mean that young leucocytes are lymphoid cells. The same might be said for the lymphocytes of the digestive leucocytosis.

Among marrow cells are forms which never reach the circulation; probably young (also old?) leucocytes and red blood-cells, and perhaps undifferentiated cells the ancestors of both series, unless one agrees with Bizzozero that none of the ancestors of red cells are without hæmoglobin. The variety of forms of cells found is so great that not one sharp dividing line can be drawn to separate a single group, and one may find evidence in favor of any ancestral tree he wishes. Two main views are held, the one that the youngest cells in the series are very large, with large, faintly staining nuclei, and protoplasm which very quickly goes to pieces, hence they are not always found; the other that they are small. According to the former, in each succeeding generation the cells become smaller and more resistant; the large fragile mononuclears develop granules and become large myelocytes, in the next generation are ordinary myelocytes, and these give rise to leucocytes. Of course, too much stress may have been laid on the shape of the nucleus as an age sign; it may be that a certain amount of irregularity in the nucleus is an expression of an amœbic cell, and that the mononuclear granular cells in some exudates are leucocytes which have resumed a resting form. The same large white cells may develop hæmoglobin in their protoplasm and become megaloblasts, the succeeding generations of which are the intermediate, then immature nucleated reds, then the mature, then the ordinary red corpuscles.

The other view considers small lymphoid elements, some even like naked nuclei, as the youngest cells, from which, by increased size and differentiation, myelocytes and erythroblasts arise, then to again diminish in size with advancing age.

Most will now agree that there is a large group of indifferent cells which will develop in whatever direction (red or white) necessity demands. The only question is, Which are these cells?

The development is more by "steps" than by a gradual transition, and those of each step are able to produce others of their kind as well as those of the succeeding generation (in point of size, etc.). The picture is still more complicated, since the line of descent of these cells is not single, but new ancestors can be found at each step in the progress, so that to trace backward is more like following a stream toward its source. It is a single river at its mouth, but as we go toward its source many tributaries are found which contribute to its volume. Thus Pappenheim traces normoblasts from small lymphocytes, megaloblasts from large lymphocytes, and considers the polychromatophilic group evidence of the transformation from a basophilic lymphocyte to a red cell; subsequent workers trace normoblasts from megaloblasts. Normoblasts certainly can produce normoblasts, and megaloblasts megaloblasts. Again the granulation may appear in cells with nuclei at various stages of deformity, as if the changes in the nucleus from round to polymorphous bore little parallelism to the development of granules.

We cannot here take up the question of the origin of leucocytes and perhaps red cells in other organs. The above remarks are not intended as a *résumé* of the subject, but an answer to many of the questions suggested to students by the study of bone-marrow. We merely mention Nothnagel's case of general osteosclerosis, with the entire marrow practically functionless, yet a normal count of neutrophiles; also the presence of mononuclear granular cells in areas of inflammatory infiltration.

4. Pigmented cells, often absent.

5. Giant-cells.

(*a*) Megalokaryocytes with one large irregular coiled nucleus. "Giant-cells with budding nuclei."

These are the "hæmatoblasts" of Foa and Salvioli, which they say give rise to smaller hyaline cells, which develop hæmoglobin to form nucleated reds. These cells are seldom found in the stained specimen, although masses of detritus which one may suspect to arise from them are found. These cells occur in the circulation in a leucocytosis, and are filtered out in the lung (see Plate II, 11).

(*b*) Multinuclear cells. Osteoclasts.

Many cells are seen, especially in fresh specimens, with very interesting degenerations and inclusions. Some large mononuclear cells contain large globules or droplets resembling myelin, droplets about 3 microns in diameter, rather uniform in size, and with the yellowish shimmer of the myelin droplets of the sputum cells. Some cells are filled with very large granules with the color and refractility of α granule (see Fig. 115, e). Such cells Howell found in the marrow of the cat in good numbers, and considers them to play an important part in metabolic changes in the marrow. In other cells occur globules of fluid, giving them a vacuolated appearance. Large "dropsical" projections both of protoplasm and of nucleus are also seen.

Unlike the red cells, in the leucocytes it is much easier to trace the degenerations. In normal blood practically all the leucocytes are normal, but when there is a leucocytosis or especially in the leukæmias many cells are seen concerning the death marks of which there is little doubt. The lymphocytes are almost devoid of protoplasm, the nucleus small pycnotic and indented, or even polymorphous (Rieder's cells) (Plate II, 17). The polymorphonuclear granular cells have nuclei very pycnotic and fragmented, although probably a chromatin thread always connects the fragments. For an interesting manner in which the neutrophile leucocytes may be classified, based on the number of nuclear fragments, and the use to which such a classification may be put, see the publications of Arneith.[44]

[44] Zeitschr. f. klin. Med., 1904, Bd. 54, p. 232.

Concerning the large pale nuclei without protoplasm there is doubt, since they could be the very sensitive young cells destroyed in the preparation of the specimen. But the similar changes in the small mononuclears, which may in leukæmia be a feature of even the majority of the cells, suggest very strongly that they are degenerations.

Late in leukæmia it would seem (said Ehrlich) as if the ability to develop neutrophile granules was lost, hence the clear cells otherwise resembling the granular cells.

All these questions would be much less interesting if the cells have the ephemeral history which some ascribe to them. Winternitz (quoted from Grawitz) estimated that in the dog the lymph supplied the blood through the lymph-duct daily a number of lymphocytes equal to more than half the total number in the body. If this be true, the chief function of most of the cells must be to increase the proteid content of the plasma. A similar question is the source of the pus-cells in cases with great pus formation, as cystitis and bronchitis or bronchiectasis, in which by actual estimation the person loses daily a number of white cells almost equal to the total number in his circulation at any one time.

Fœtal Blood.—In the three-months' human embryo Engel found nucleated red cells of normal and large size, "metrocytes of II. Generation"; that is, large spherical nucleated reds, 12 to 20 microns in diameter, rich in protoplasm, the nucleus relatively small, 3.5 to 6 microns in diameter; but in some cells 17 to 20 microns in size the nucleus was 7 to 8 microns. These cells occurred in frequency of from 4 to 6 per 100 normal reds. (Metrocytes of I. Generation he describes from mouse embryo's blood as spherical cells from two to three times the size of a normal red cell, the nucleus often in mitosis and filling but a relatively small part of the cell; this, he says, is not a megaloblast nor a gigantoblast. At this stage there are no non-nucleated reds and no leucocytes.) At this age occur two forms of normoblasts,—those staining orange, from 5 to 9 microns in diameter and the nucleus 3.5 to 5 microns; those staining red (Ehrlich stain), about 7 to 8 microns in diameter, with a relatively large nucleus rich in structure 5 to 6 microns large, the protoplasm scanty and ragged; in this latter group are some large cells 16 microns in diameter and a nucleus of 11 microns; these are Ehrlich's megaloblasts.

The other cells were free metrocyte nuclei, lymphocytes, neutrophile myelocytes, and leucocytes.

In embryos of 6 cm. length the non-nucleated reds were to the nucleated as 12:1; of 12 cm. embryo, 55:1; of 16 cm. 150:1; of 19 cm. 176:1. In the 6 cm. embryo the metrocytes were 4 per cent. of the reds; in the 12 cm., 0.25 per cent., and later none. The leucocytes in the 6 cm. embryo were to the reds as 1 : 500 to 1000.

Engel admits that embryos of the same age differ so that he could not tell age from the blood.

We have had opportunity to study the blood of a fœtus 15 cm. long, and found red cells, 1,168,000; hæmoglobin, 25 per cent.; leucocytes, 9000. Nucleated reds, 1 : 19 of total reds, normoblasts and intermediates, beautiful polychromatophilia.

In an embryo 20 cm. long we found reds, 2,652,000; leucocytes, 28,000; hæmoglobin, 38 per cent.

In an embryo of 23 cm. Engel found the reds (heart's blood), 3,300,000; hæmogobin, 80 per cent.; leucocytes, 40,000. Nucleated reds were to non-nucleated as 1 : 120, and all normoblasts. Of the leucocytes, the granular were to the non-granular as 2 : 5; neutrophile myelocytes and leucocytes present with all transitions, and a few eosinophiles.

The blood of a 27 cm. embryo contained nucleated and non-nucleated reds in relation of 1 : 200, leucocytes to erythrocytes as 1 : 90, polymorphonuclears to mononuclears as 4 : 5.

Leucocytosis.—By this term was meant an increase above normal of the white cells of the blood, but the term is now used of a transi-

tory, symptomatic, absolute increase of the polymorphonuclear neutrophiles especially, in the peripheral blood, above the maximum that is normal for the individual in question in the condition in which he at that time finds himself.

In general 10,000 leucocytes per cubic millimetre is the limit an increase above which is said to be pathological. But the matter is a relative one, and only when so considered does the condition have the clinical value claimed for it. Some persons have normally a leucocyte count of 10,000 to 12,000. The count also depends on the condition of the person. For instance, if cachectic with a leucocyte count of 4000, a rise to 8000 would mean as much as a rise to 20,000 would for some normal persons. This was beautifully exemplified in one case of typhoid fever with a leucocyte count of 1600. A parotitis developed and the leucocytes promptly rose to 3200, a true leucocytosis for that person at that time.

It is also transitory and symptomatic, which separates it from leukæmia.

It now has a more special meaning and is used only of an absolute increase of the polymorphonuclear neutrophile cells, while an increase of the various other types of white cells bears a special term according to the cell increased; for instance, if it is the mononuclear non-granular cells, lymphocytosis; if the polymorphonuclear eosinophiles, eosinophilia; if the mononuclear granular cells, myelæmia, etc. It is very seldom that one group of cells alone is increased; usually others are to a less degree; but since there is evidence that the various cells are not all related very closely at least to one another, it is their absolute number which is to be considered rather than their relative, that is, than their percentages or "formula." With even a diminished per cent., providing the total count be raised, the absolute number may have increased, while the reverse also is true that when the percentage seems to indicate an increase the absolute number may have dropped if there is a diminution of the total number.

Or a group of cells may remain unchanged, while other groups change much. A good illustration of this is the following, a case of Frazier and Halloway: Count, 13,040; polymorphonuclears, 78.2 per cent. (*i.e.*, 10,197); small mononuclears, 16.8 per cent. (2191). The total count rose to 54,960; polymorphonuclears, 90.4 per cent. (49,684); small mononuclears, 4 per cent. (2198).

The "general type" of leucocytosis—*i.e.*, an equal increase of all the leucocytes—is rarely seen. It results from stasis of blood in the capillaries, following a cold bath, or massage,—*e.g.* Also cases of the digestive leucocytosis and that of pregnancy, *et al.*, show it in some degree.

Classification (Limbeck).—1. Physiological: (*a*) Digestion; (*b*) Pregnancy; (*c*) Newborn. 2. Pathological: (*a*) Inflamma-

tory; (*b*) Malignant tumors; (*c*) Post-hemorrhagic; (*d*) Agonal. 3. After medicinal and therapeutic measures. 4. Various other causes, as shock, etc.

Digestion Leucocytosis.—The leucocytes of a normal person who after a fast of twelve or more hours partakes of a rich proteid meal will usually rise to about one-third above the normal number. The count begins to rise in about one hour as a rule, reaches a maximum in from three to five hours, and then decreases. While the polymorphonuclear neutrophiles are especially involved, the small mononuclears are to some extent, in some cases considerably. For some persons no preliminary fast is necessary; in others the leucocytes do not rise at all. (Limbeck thinks habitual constipation explains the latter.) Children show it more markedly than adults, and the well nourished than the poorly nourished; it is greatest in the infant after his first meal of cow's milk. For the nursing infant it is said to be absent, and hence the opinion (Moro) that it is a reaction against foreign proteid. A rich proteid meal is necessary, hence diabetics show it well. Particular stress should be laid upon this point, that the meal should be unusually large, for the leucocytes are even fewer after a light meal (also after some heavy ones), or again they may not change. It is absent in the herbivorous animals, and little in man after a vegetable meal.

The explanation is in doubt. One thing is quite certain, that it is due to the absorbed products of proteid digestion, which have a positive chemotactic influence. Hofmeister suggests the proliferation of the large masses of lymphoid tissue along the intestine, due to the stimulation of the digestive processes, to be the cause; hence it is a mixed leucocytosis. The lymphocytosis is, however, not always present.

Jaffé says that in children the leucocytosis is not dependent on the meal, but is periodic.

The reverse relation is also true. Persons in starvation show a low leucocyte count; Succi, who fasted seven days, had a count of 861 per cubic millimetre, while the insane with melancholia often have counts below 3000. On the other hand, well-nourished persons often have counts from 10,000 to 12,000.

The function of the leucocytes is probably not alone protective, but they play an important part in absorption, transportation, and assimilation of food; hence their number depends much on the age and nutritional condition of the person.

There is some value in the digestion leucocytosis to aid in differentiating between pernicious anæmia and cancer of the stomach. In severe blood diseases, pernicious anæmia, and in ulcer and other gastric diseases it is present, while in cancer of the stomach even fairly early, it is sometimes absent, but not always. It is absent in

some benign conditions.[45] Gastric catarrh and involvement of the lymph-glands are given as its explanation. We wish to emphasize that a rich proteid meal must be given these cases and the leucocytes counted once an hour. Only a considerable rise is of value.

Leucocytosis of Pregnancy.—About 75 per cent. of women during the last months of pregnancy show a count above normal, an average about 13,000 per cubic millimetre. This is especially true of primiparæ, and yet it would seem to be more the youth and the nutritional condition of the patient than the fact that she has had no previous pregnancies. The count rises until the end of pregnancy, and then disappears in from four to fourteen days after delivery. The differential count may remain practically normal, yet it is the polymorphonuclear neutrophiles that are especially involved. In multiparæ there is also a rise, but it is within physiological limits.

The explanation has been disputed. It is agreed that it is not the pregnancy *per se.* V. Limbeck considers it a prolonged digestion leucocytosis due to the additional need of nourishment for the mother and child, in favor of which is the absence of a digestion leucocytosis or even a diminution, which is explained by assuming that the leucocytes migrate to the placenta, where is the greatest accumulation of the positively chemotactic products of digestion. The condition of the breasts is also suspected; others say an overaction of the lymphatic system. But the view most commonly held now is a slight autointoxication, against which the primipara reacts better than a multipara. Thomson found that of 33 counts on twelve pregnant women made during the eight months of pregnancy but one was below 7000; the highest 13,200. In these few cases there was no rise toward the end, but the counts were practically the same for the second month as later.

But the question is, What is the usual count for a normal woman? is it 5500? if so, pregnancy causes in all cases a relative rise, and in most an absolute leucocytosis.

Zangemeister and Wagner[46] think the question not quite so clear. Of 47 normal non-pregnant women, all under practically the same conditions, from twenty-one to thirty-four years of age, 35 (74 per cent.) had a count above 10,000 (mean about 12,500). The leucocytes of pregnant women (57 cases) varied within the same limits as non-pregnant (70 per cent. above 10,000; mean count between 12,500 and 15,000), nor did the number of previous pregnancies seem to make any difference. The counts which these writers report are about the same as those of the writers claiming a leucocytosis as a feature of pregnancy, only the former claim that normal non-pregnant women give the same. During labor, of 63 cases there was a rise even to three times the previous count in nearly all cases, with the maximum at or just after delivery. This was especially marked in cases of prolonged labor or of those who suffered greatly. In quick, easy labors the rise is insignificant.

In 75 cases during the puerperium there was a rapid decrease to normal. On the seventh or eighth day an increase of mononuclears, with the involution of the uterus (Rouslacroix and Benoit). The study of two cases of version led them to think the cause of the rise was the contractions of the uterus.

In pathological cases the leucocytes give no aid in diagnosis or prognosis, since as high counts are seen in the physiological cases.

Lobenstein[47] considers that there is a leucocytosis of pregnancy, the average of 50 cases during the ninth month being 11,854 for primiparæ, and 9346 for multiparæ; and on the third day of the puerperium, 13,200 for primiparæ, and 11,600

[45] Rencki, Arch. f. Verdauungskr., Bd. vii.
[46] Deut. med. Wochenschr., July 31, 1902.
[47] Am. Jour. Med. Sci., 1904, vol. cxxviii. p. 281.

for multiparæ. These figures are too nearly normal to name them leucocytoses. In 20 cases the digestion leucocytosis was tried, found present in 13, but an actual diminution in the count in 6. Of 13 cases of eclampsia, in 6 mild cases the highest count was 31,000; in 6 severe, 40,000 to 50,000; and in one severe case, 106,000 and death. He concludes that the leucocytosis is roughly parallel to the degree of intoxication and to the resistance. A low count and a rapidly falling count are bad signs.

Leucocytosis of the Newborn.—Although there are relatively so many blood-building organs in the fœtus there is leucopenia, since as yet there is no function for the leucocytes (Askanazy). The statement usually made is that at birth there is a leucocytosis of from 17,000 to 21,000, and after the first feeding a rise to from 26,000 to 36,000, with the increase chiefly in the number of small mononuclears. Examination of the infant's blood exactly at birth, however, in case the teacher wishes to demonstrate a true lymphocytosis, will assure the disappointed one that this is by no means the case, and he will usually find a condition of the leucocytes quite like that of the adult. The question has been studied by Gundobin, Carstanjen, and Warfield,[48] with the following results. On the first day after birth the average leucocytosis is about 26,000 (11,700 to 34,700); on the third day, the average is 13,270, and on the eleventh day 15,740. For the first few days there is an absolute increase in the number of polymorphonuclear neutrophiles, with a percentage of 70.42 on the first day, 53.16 on the third, and 34.2 on the eleventh. The large mononuclears and transitionals are high, being 10.76 per cent., 16.67 per cent., and 15.98 per cent. respectively on these three days. The eosinophiles vary much; Mastzellen and myelocytes are few and rare. It is not until the eleventh day that the count which is usually considered normal for infants, with 40 per cent. small mononuclears, appears.

This high count of the leucocytes has been explained by a concentration of the blood or a digestion leucocytosis, but the more rational explanation is the rapid blood formation at that age. Although normal infants vary much, yet this rather high count may continue until from the third to the sixth year, after which time the blood picture of the adult prevails. During these early years the polymorphonuclear neutrophiles vary from 18 to 40 per cent., the small mononuclears from 40 to 60 per cent. of the total number, and often there is a slight increase in the eosinophile cells.

Leucocytosis of Inflammations and Various Febrile Diseases.—There is an absolute increase of the polymorphonuclear neutrophile cells especially accompanying most inflammations, most acute infections, and other febrile diseases, which is roughly parallel to the temperature, and which depends especially upon the activity of the inflammatory process and the condition of the patient.

[48] Amer. Medicine, September 20, 1902.

The following general statements may be made. Whatever the immediate cause, a leucocytosis represents the reaction of the individual to the disease. In those conditions usually accompanied by a leucocytosis a high count means a vigorous reaction, little more; a low count may mean a poor reaction, hence indicate a poor prognosis, or the infection may be of so mild a degree that it can elicit little or no reaction.

On the other hand, diseases differ in their ability to produce a leucocytosis: some do practically always, as pneumonia, and in a degree roughly parallel to the virulence; some never, as measles, malaria, and tuberculosis; some perhaps an early leucocytosis followed by a leucopenia, claimed for typhoid fever, but doubted by most; some none at first, then a rising count, as typhus fever, some cases of influenza, and smallpox. Some diseases ordinarily without leucocytosis may in cases of great severity show one, as malaria. In certain cases much depends on the situation of the infection, as in typhoid fever, which infection, when it causes empyema or periostitis, is accompanied by a rise of leucocytes; also tuberculosis of the meninges, and caseous pneumonia.

In cases of local infections, as abscess formation, the leucocytosis is a symptom related to the fever and other toxic features, and evidently like them caused by, and its severity determined by, the toxine absorbed; for following operation and free drainage both quickly drop to normal. For much the same reason the count runs quite parallel to the richness of the exudate in pus-cells.

It is not the exudate formation alone which governs the leucocyte count, for cases with free drainage of pus may lose enormous numbers of white cells daily (almost as many as are in the circulation at any one time), and yet show a normal count. This is well seen in some cases of chronic bronchitis, bronchiectasis, cystitis, etc., in various bone and joint abscesses with discharging sinuses, in empyema after operation, etc. The agent causing the leucocytosis seems the same as that causing fever, for they usually begin and end together, depending on the free drainage of the exudate.

Of course one would expect that a great loss of cells in an exudate would mean a diminution in those of the blood, and in acute cases, a spreading peritonitis for instance, this is thought to be the explanation of the sudden drop in the count of the blood.

In general, the leucocyte count runs in no way parallel to the severity of the condition; a simple local felon may cause as high a leucocytosis as an appendix abscess, and a fatal pneumonia as little rise as a boil.

Among the conditions causing leucocytosis are: *Acute lobar pneumonia,* the best studied (page 567).

Acute tuberculous pneumonia (page 562).

Acute articular rheumatism (page 571).

Diphtheria (page 561).

Acute cerebro-spinal meningitis caused a leucocytosis in all of 21 cases (Osler); in 4, over 40,000; the highest, 47,000. The leucocyte count is of no especial value in distinguishing the various forms of meningitis, since it is also present in the tuberculous.

An ordinary acute follicular tonsillitis usually causes a leucocytosis. This was true of 18 of 26 of our recent cases (12, from 10,000 to 15,000; 3, above 20,000; the highest, 27,000). There was considerable fever in all the cases with high counts.

Scarlet fever (page 561).

Mumps.

In whooping-cough the leucocytes, especially the lymphocytes,

are increased three or four times the normal amount, averaging 40,000, the degree of leucocytosis depending on the severity of the case and its complications. It is more pronounced the younger the child is. The early appearance of the leucocytosis is important in diagnosis. The rise is chiefly of the lymphocytes, but not entirely. It begins with the disease, during the catarrhal stage, and continues longer than the paroxysms, is maximal during convalescence. Others claim it is a true leucocytosis; again others, that the formula is little disturbed.

Rabies sometimes causes a true leucocytosis of even 25,000, with 98 per cent. pmn. n.

Erysipelas causes a leucocytosis which runs fairly parallel to the temperature, of 10,000 to 20,000 in mild cases, 20,000 to 30,000 in more severe. Its polymorphonuclear neutrophile character is more marked in adults than in children. These cells may be 92 per cent. in fatal cases. As the count falls, the eosinophiles may rise considerably.

In 6 cases the leucocytes were normal in 2, moderately elevated in 2, and 26,000 and 34,500 in the other two. The red cells were normal in all.

In acute ulcerative endocarditis the leucocytes are high as a rule, especially in those cases running a protracted course, an important point in diagnosis, any long continued leucocytosis suggesting this. In rapidly fatal cases there may be no rise.

In 6 cases recently at death the count stood 7070, 13,600 (it had fallen from 34,000), 17,000, 47,000, and 48,000 (it had risen from 9800). In another case, 12,000.

In intestinal obstruction the leucocytes rise rapidly to about 16,000 when partial, to 20,000 or more when complete; with over 20,000 cells within the first twenty-four hours the chances are in favor of gangrene. This rise of leucocytes may be of value in a case of suspected post-operative obstruction. (Bloodgood.)

Following a thyroidectomy the myxœdema is accompanied by a count of even 49,000.

Smallpox (page 561).

Cholera.—At the algid stage the leucocytes may number from 40,000 to 60,000, and rapidly disappear during the stage of reaction.

Pyogenic inflammations of the serous membranes, meninges, pleura, pericardium, peritoneum, not tuberculous, are accompanied by a leucocytosis which bears some relation to the cellular richness of the exudate in leucocytes, more to the fever. The count varies with the progress of the disease, since it may drop to normal while the process is stationary even if the temperature remains elevated, until a slight

spreading of the process causes a rapidly rising count. This is well seen in pelvic inflammations.

In 99 cases of PLEURISY WITH EFFUSION the red cells were practically normal; in 65 the leucocytes were below 10,000 cells, and in but three of the remaining were they over 15,000. Cabot reports almost exactly the same figures for the Massachusetts General Hospital (314 cases; 33 per cent. above 10,000; 6 per cent. above 15,000). The low counts are interesting since so many such cases are clearly tuberculous.

In EMPYEMA, on the other hand, there is almost always a leucocytosis, except in cases (14 per cent.) allowed to remain without operation for some time.

In 37 cases of ACUTE FIBRINOUS PLEURISY the leucocytes varied from 10,000 to 22,900 in 24. In the rest the count was normal.

INFLUENZA is a term applied to a wide group of cases, but with the diagnosis seldom confirmed by cultivating the organism. The demonstration that many cases of bronchiectasis and chronic bronchitis are really la grippe throws considerable doubt on the figures given of the blood-findings. But accepting the diagnoses as they stand, the leucocytes are normal in about two-thirds of the cases (Cabot), moderately increased in the rest. Blum [49] states that in the typhoidal or abdominal form there is leucopenia. Gerber [50] states that the leucocytes rise not at the height of the disease, but as the fever falls; that a count of 20,000 cells indicates pneumonia. During the rise the eosinophiles decrease or disappear.

In almost half of our cases the count was above 10,000 at the height of the disease, reaching even 25,000. What is of more interest is that nearly all the cases in which several counts were made showed early a very low count, then a sharp rise, which fell after the temperature was normal. This may explain why in the cases with but one count the leucocytes may be low, even 3000 to 5000 when the temperature is 100° to 105°, and high when the temperature is normal. It also shows that for diagnosis it is not one count that is of value, but the leucocyte curve.

Any *pyogenic processes of mucous membranes* accompanied by fever may cause a leucocytosis, as enteritis, urethritis, etc.

ACUTE BRONCHITIS is accompanied by a leucocytosis which continues as long as the fever. The count was from 10,000 to 20,000 in 30 of our 67 cases.

In CHRONIC BRONCHITIS the emphysema and attending cyanosis may explain the few cases with slight leucocytosis, present in just half of our cases.

The red counts averaged high, the mean being 5,000,000. Of 25 cases, 3 were above 7,000,000 (maximum 7,900,000).

In a case of true FŒTID BRONCHITIS, 22,500.

In 11 cases of BRONCHIECTASIS the leucocytes were 20,000 in 2;

[49] Wien. klin. Wochenschr., 1899.
[50] Wien. klin. Wochenschr., 1900.

between 10,000 and 20,000 in 4; normal in the others. The 6 with leucocytosis ran a slight temperature.

Among the local pus processes in which the leucocyte count is an advantage are *appendicitis* (page 571), *pelvic inflammatory disease* (except tuberculous, and mild in gonorrhœal), *abscess of the liver, empyema of the gall-bladder, ovarian abscess, abscess of the brain,* etc.

In ABSCESS OF THE LUNG the counts have been reported very high, even 60,000; or low. In 3 recent cases they were 8100, 12,300, and 12,500.

In two recent cases of GANGRENE OF THE LUNG the leucocytes numbered 20,000 and 48,000.

In 25 cases of GONORRHŒAL ARTHRITIS, the mean red count was 4,500,000; lowest, 3,600,000; the mean leucocyte count was 9000; 8 of 23 cases were between 10,000 and 20,000.

In PERIRENAL ABSCESS, 5 cases, the leucocytes varied from 19,000 to 36,000; PYELITIS, 4 cases, the leucocytes were from 10,600 to 19,500.

In PYELONEPHROSIS, 2 cases, 18,000 and 28,500; HYDRONEPHROSIS, 2 cases, 6400 and 9000; pyelonephritis, 1 case, 8000.

In RENAL CALCULUS, 4 cases, the leucocytes during the colic were from 12,000 to 18,000.

In GOUT the red cells are practically normal, and 5,000,000 or over in but 2 cases (of 13 cases the lowest was 4,300,000). The leucocytes rise with the onset of an acute joint attack. (In 18 cases there was a mild leucocytosis,—10,000 to 14,000 in 7 cases.) In a case counted daily, simultaneous with the rise in temperature and tenderness of the joints, the leucocytes rose soon to 31,000, and fell again as joint symptoms subsided.

There is a leucocytosis in DIABETIC COMA and in URÆMIA.

The question of a POST-OPERATIVE LEUCOCYTOSIS which is not due to infection is very important from a surgical point of view, in the diagnosis of sequelæ to an operation.[51] King[52] considers that a curve with an increase of 5000 to 10,000 cells during the first six to thirty-six hours is a normal post-operative condition, provided the rise is not longer sustained. Others give the limits as from thirty-six hours to five days. It reaches its maximum during the first twelve hours. The height bears no relation to pulse or temperature. If the rise is over 10,000 cells, and is sustained longer than a few hours, it is very suspicious. The normal ante-operative count for each case should always be determined before operation. The highest count

[51] Frazier and Halloway, Contrib. from the Wm. Pepper Lab. of Clin. Med., 1902, No. 3.

[52] Am. Jour. Med. Sci., September, 1902, vol. cxxiv.

was 26,300, six hours after the operation. The nature of the operation seemed to have little influence on the count, yet the height of the rise is roughly parallel to its extent and character. The highest count was in a nephrotomy, 32,000 cells. King found in no case a rise of 20,000. There is little relation between it and the post-operative fever. Chloroform anæsthesia can cause a true leucocytosis, but this is very transitory indeed; ether none.

In point of degree there is no sharp line between the leucocytosis of infected and non-infected wound repair, but the latter is on the wane at a time when the former is just beginning.

When a packing is changed the leucocytes may rise somewhat. In a closed wound the leucocytes are a good index of an infection.

The diseases causing, as a rule, no leucocytosis are *typhoid fever* (page 565), *measles* (page 560), and *tuberculosis* (page 562).

Pseudoleucocytosis.—Certain other blood-changes occur in much the same conditions and are supposed to have the same significance as a leucocytosis. Among these are iodophilia (page 503) and a relative increase of the polymorphonuclear neutrophiles while the total count does not rise above normal. This is seen in cancer, septicæmia, etc. Also degenerations of the leucocytes, fragmentation of the nuclei, as in cancer, the appearance of myelocytes, etc., indicate much the same.

Leucocytosis of Malignant Tumors.—Cases of carcinoma (page 578) and sarcoma (page 581), while not always yet often, present a leucocytosis which bears no relation to the kind of tumor, except that it is more common with the sarcomata than with carcinomata. There is none in epithelioma of the skin, while in the case of some organs, for instance gastric carcinoma, it is common. On the whole it bears no relation to the situation of the tumor. The blood of a case of sarcoma has been described as even simulating a leukæmia. It is a leucocytosis of polymorphonuclear cells, and mononuclears in some cases as well, which disappears after the removal of the tumor. The occurrence of the leucocytosis in these cases is so variable that it certainly is not alone the presence, nature, or situation of the tumor which determines it.

Post-Hemorrhagic Leucocytosis.—After a large hemorrhage there is a rise of the leucocytes which begins in from ten to fifteen minutes, and in one hour reaches about 16,000 to 18,000. This lasts a few days and then disappears. It is an increase of the polymorphonuclear neutrophile cells. The cause cannot be a new production of cells, since it begins so suddenly; it is best explained by the tissue lymph which flows into the vessels in order to restore the volume of blood, carrying with it a large number of white cells. Many consider that the nature of the wound is important, since often with injury and without

hemorrhage there is a leucocytosis, while if the hemorrhage be the result of very little injury, as, for instance, in case of a gastric ulcer, the duration of the leucocytosis is very brief, even but two days. Stassano and Billou [53] found a hypoleucocytosis to follow a severe hemorrhage; a true leucocytosis smaller losses of blood.

In a case of cirrhosis of the liver with fatal hemorrhage from the stomach, before death the reds were 1,960,000, hæmoglobin 23 per cent., leucocytes 23,000.

Agonal Leucocytosis.—Belief in an agonal leucocytosis existed before the inflammatory leucocytoses were understood, and hence many cases may have been those of terminal pneumonia. Yet this does not explain all of the high counts at the last of a disease. Cabot's case, for instance, of pernicious anæmia resembled a leukæmia. Such cases are rare, it is true. In most diseases the leucocytes do not change or even drop at death, while in some cases they do rise, which Ehrlich ascribes to the slowing of the circulation and hence the accumulation of the leucocytes along the periphery of the blood-vessels. Arneth doubts an agonal leucocytosis, thinking the leucocytoses which occur then are easily explained by the disease causing death.

Medicinal Leucocytosis.—After the administration of any of a long list of drugs, including the ethereal oils, tonics, mýrrh, turpentine, peppermint, whether by mouth or subcutaneously, there may result a considerable rise of the leucocytes. In the case of the drug by mouth it seems to be comparable to a digestion leucocytosis, while in the case of subcutaneous injection the local reaction also may be important. The list of these drugs is so long and varied that an enumeration is not valuable. It is interesting that the extracts of certain body tissues and organs seem positively chemotactic.

The reverse is also true, as in the case of blood poisons which destroy the cells, hence with phenacetin, the chlorates, and pyrogallic acid there is even a drop.

Other Causes.—In the case of animals simple violence will cause a rise of the leucocytes. In man, hard work, severe sweat, heat and cold, will also; many vasomotor influences, slowing of the circulation, as for instance by cold, Thayer finding in typhoid fever that a cold bath, especially those leaving the patient shivering, would raise the count about 6000 cells; the formula remained the same.[54] In any cyanotic part the leucocytes are increased.

Violent exercise will cause leucocytosis, as was seen in the runners of a twenty-five mile race whose leucocytes rose from 14,000 to 22,000.[55]

[53] Compt.-rend. Soc. Biol., 55, p. 180.
[54] See also Becker, Deut. Arch. f. klin. Med., 1901.
[55] Larrabee, Jour. of Med. Research, 1902, vol. ii.

Mixed Leucocytosis.—By this is meant an increase of amœboid and non-amœboid cells, that is, of granular and non-granular; but as commonly used it means the presence in a leucocytosis of neutrophile myelocytes. The best known condition is in leukæmia, in which the absolute number of myelocytes may be from 50,000 to 100,000. In no other condition does the absolute number of myelocytes rise above 1000 (Ehrlich). Leukæmia is the chief condition in which the eosinophile myelocytes are found, and where Mastzellen are increased. All forms of non-granular cells are also increased. The next most important condition is pernicious anæmia. Almost any leucocytosis could be called mixed, since it is common to find a few myelocytes, young cells swept out too early. In this case it has no significance if the cells disappear as the count falls, but should they remain after the count sinks it means exhaustion of the bone-marrow (that is, if the septic features continue). The appearance of myelocytes in those conditions means more,[56] provided we grant that the leucopenia of such cases (typhoid fever, *e.g.*) is proof of the inhibiting action of the bacterial toxine on the marrow, which is by no means certain.

In cancer of the bone-marrow, sarcoma, and metastatic carcinoma, there is a mixed leucocytosis which some suppose is due to the negative chemotaxis of the tumor. In severe post-hemorrhagic anæmias and in various children's diseases, especially diphtheria, anæmia, rickets, congenital lues, and pneumonia just after the crisis, it also occurs.

Mastzell Leucocytosis.—The only condition in which these cells are increased in the blood is splenomyelogenous leukæmia, in which case they may be even 15 or 20 per cent. of the increased count. Isolated cases, as of cancer, septic bone disease, various skin diseases, and even chlorosis, have been reported.

Increase in Large Mononuclears.—The origin of these cells is unknown. They are increased absolutely in typhoid fever, post-febrile measles, and especially in malaria in which they are in large numbers, even 20 to 30 per cent. of an almost normal count, a point of diagnostic value.

Lymphocytosis.—Ehrlich classified increased leucocyte counts as active, passive, and mixed leucocytoses. A true leucocytosis is active because amœboid cells are increased which are supposed to have wandered out into the circulation in response to a positive chemotactic agent. A lymphocytosis he called passive since he supposed these cells to be mechanically washed out of the lymphatic tissue.

That some lymphocytes are amœboid on a rather hot stage (44° to 46° C.) is granted; that they can migrate into the tissues in certain skin diseases is also

[56] Schindler, Zeits. f. klin. Med., 1904, Bd. 54, p. 512.

granted; they may be the cells of a pleural exudate; yet there is little resemblance between the true leucocytosis and the lymphocytosis which would lead us to call the latter active.

The term lymphocytosis always refers to the absolute number of these cells.

Physiologically the condition exists in infants, and during a digestive leucocytosis. Pathologically it occurs in simple gastro-intestinal disturbances of children (page 574), in whooping-cough (page 492), cervical adenitis, the reaction to tuberculin, malignant lymphomata, sarcoma multiplex cutis. The best illustration is lymphatic leukæmia, in which case over 90 per cent. of the 140,000 cells may be small mononuclears. There is an absolute increase in splenomyelogenous leukæmia, and after splenectomy there is very constantly a slow increase of lymphocytes to even twice the normal number, which begins in about a month and continues during the first year. The blood picture may even suggest a leukæmia. The same, but to a less degree, occurs in chronic spleen tumor.

The leucocyte count may be low and yet a true lymphocytosis exist, as in a recent case of amœbic dysentery, with a count of 2500 cells, 68 per cent. of which were small mononuclears. The best illustrations among the chronic disease are hereditary lues and severe rickets. The statement is usually made that in chlorosis, pernicious anæmia, debility, late typhoid, Graves's diseases, hæmophilia, scurvy, and during thyroid treatment there is a lymphocytosis, but in many of these cases the increase was not true but relative, since the granular cells were diminished.

The leucocytosis of children is sometimes a marked lymphocytosis, as in the case with enlarged tonsils mentioned by Churchill, in which the count was 20,000, 70 per cent. of which were small mononuclears. In the diagnosis of lymphatic leukæmia these cases must be remembered. The clinical microscopist must also remember that there is almost no apparent relationship between enlarged lymph-glands and lymphocytosis, as is seen in Hodgkin's disease, chronic, and acute lymphatic leukæmia.

One interesting case Ehrlich mentions of general lymphosarcoma had but 0.6 per cent. small mononuclears in the blood.

Leucopenia results from the reduction in one group of cells or a general reduction of all. The former is seen during typhoid fever. Below 5000 cells per cubic millimetre is usually considered a leucopenia. As example, it is claimed that the first step in a leucocytosis is a drop due to the disintegration of some and accumulation of other white cells in the internal organs, liver, lungs, spleen, followed by a rise due to the emptying of the depots of these cells. In tuberculosis of lymph-glands the count is even below 600 cells.

Cases have been reported under the name "alymphæmic lymphomatosis," as one case (Schwarz) with fever, acutely swollen glands, and only 600 leucocytes per cubic millimetre, and they all lymphocytes (no autopsy). Türk said some such cases show no features of an infection.

The relationship between abdominal troubles and leucopenia is interesting. Typhoid is a disease with leucopenia while limited to the intestine, with leucocytosis when other organs are involved; tuberculous peritonitis uniformly causes no leucocytosis; "abdominal influenza" causes no leucocytosis.

Following some fevers the count is low, as typhoid with but 2000 cells. In a case of hæmoglobinuria here the count of leucocytes ran nearly parallel to that of the red cells, with, at the lowest, 2,500,000 reds and 950 leucocytes.

In cases of starvation or malnutrition due to any cause the count is low; *e.g.,* starvation, voluntary (page 489), or due to disease as in cancer of the œsophagus (page 580). In one of our cases of ulcerative colitis the patient's red count was 2,100,000, leucocytes 700 per cubic millimetre.

In acute miliary tuberculosis (page 564) the counts are very low sometimes, with a great reduction of young cells. In all chronic intoxications, alcohol, morphia, lead, ether, mercury, arsenic (hence the drop in leukæmia?), leucopenia is the rule.

Eosinophilia.—By eosinophilia is meant an absolute increase of the eosinophile cells. The average percentage in a normal case is from 2 to 4 per cent., and while often it is the percentage by which the eosinophilia is judged the term should be limited to those cases in which the absolute number of these cells is above 250 per cubic millimetre.

Those conditions in which these cells are increased vary so much that they are well termed the most capricious cell of the blood.

I. There is a PHYSIOLOGICAL EOSINOPHILIA during childhood.

II. **Diseases of the Hæmatopoietic Organs.** 1. BONE-MARROW.—(*a*) *Splenomyelogenous leukæmia* is a diagnosis which Ehrlich says should be made only in case there is absolute increase of these cells. As a rule, they are much increased, even to 29,000 per cubic millimetre (Zappert). The number of undoubted cases, however, is increasing in which these cells are very few or, indeed, entirely absent.

(*b*) In sarcoma of the bone-marrow they are sometimes present in great numbers (page 581). In (*c*) osteomyelitis and (*d*) osteomalacia they are sometimes increased.

2. SPLEEN.—One year after extirpation of the spleen there slowly develops an eosinophilia which lasts for several months. These cells are increased to from thirty to fifty times their normal number, and may

constitute even 36.6 per cent. of the leucocytes. A somewhat similar condition is present in cases of chronic splenic tumor, in which these leucocytes may be from 7 to 12 per cent., and in tumors of the spleen; in both cases the spleen is partially functionless.

3. Lymph-Glands.—An eosinophilia accompanying disease of these glands is rather doubtful, since in the cases described carcinoma metastases to the bone have not been excluded. In one case with such metastases the eosinophile cells numbered 60,000.

III. **Asthma.**—In true bronchial asthma at the time of the paroxysm eosinophile cells may be from 10 to 20 per cent. of the leucocytes; in one case of Billings, between 53 and 54 per cent. This is of diagnostic importance in excluding asthmatic attacks due to other cause. In emphysema these cells are also increased, in one case being 53.6 per cent. of a total of 8300 leucocytes.

IV. **Skin Diseases.**—A large number of skin diseases are accompanied by eosinophilia. This depends more upon the extent of the lesion than its nature. The contents of the pustules are sometimes interesting, since all of the leucocytes are eosinophiles.

In one case of pemphigus reported by Zappert there were 4800 eosinophiles per cubic millimetre of blood; in one of pemphigus vegetans in this clinic on one day, with a total of 20,400 leucocytes, 2.6 per cent. were eosinophiles; on another day, 11.6 per cent.; in pellagra and psoriasis these cells are sometimes increased; in urticaria they may reach even 60 per cent. of the total number; in a case of purpura with cyanosis in this clinic, of 52,000 leucocytes, 11 per cent. and later, with almost as high a count, 25 per cent. were these; in certain cases of eczema they are increased; of two cases of scleroderma in this clinic, in one they constituted 2.4 per cent. of 7000 leucocytes; in the other, 3.3 per cent. of 10,500; in five cases of purpura simplex the red cells and hæmoglobin were practically normal; three had a leucocytosis of from 10,100 to 40,600 (eosinophiles, 12.1 per cent.). This last case was also one of myositis with the eosinophile cells running from 11.3 to 25.6 per cent. (total leucocytes 20,300), but no other evidence of trichinosis could be found. In one of three cases of purpura rheumatica there was a leucocytosis of 13,300. In three cases of Henoch's purpura the leucocytes in one were 10,000. In two cases of purpura hemorrhagica the leucocytes numbered 7500 and 6600; of the latter 4.5 per cent. were eosinophiles. In one case of purpura hemorrhagica 9.4 per cent. of the 5200 leucocytes were eosinophiles. In one case of morbus errorum the leucocytes were 5000, the eosinophiles 18 per cent.; they slowly diminished for four counts during one month when in the hospital, at which time they reached normal. After chemical irritation of the skin, for instance by mercuric chloride, these cells are much increased, even to 14 per cent.

V. **Parasites.**—Any parasite, from the harmless pin-worm to the most malignant uncinaria, may cause an eosinophilia. It is not always present, nor does its degree bear any relation to the severity of the infection or the danger of the parasite. Amberg in amœbic dysentery of children found a slight eosinophilia.

In trichinosis, Brown demonstrated this as a most important point in diagnosis, the maximum count being a total of 35,000 leucocytes, 68.2 per cent. of which were eosinophiles. This eosinophilia is not

always present, as in the case of Howard and one of Da Costa, but was present in all the others of the 25 to 30 cases that have been reported. In a case of Gwynn's they were 65.9 per cent. In Brown's case they gradually fell. The neutrophile cells are relatively and absolutely low. He was unable to get any Charcot-Leyden crystals from the blood, hence considers some other element than this necessary for their formation.

In uncinariasis the eosinophile cells have constituted usually from 8.2 to 10 per cent., in one case reaching 72 per cent. of the count. Our highest count was 13 per cent. in a total of 7400 leucocytes. In one case with Tænia saginata the eosinophile cells were 34 per cent.; of Ascaris lumbricoides, 19 per cent.; Oxyuris, 16 per cent.; Strongyloides intestinalis, 13.5 per cent.; Bilharzia, 20 per cent. In filariasis they vary from 4 to 17 per cent.; in Calvert's case, 22 per cent., reaching a maximum at day (some say night). Calvert thinks that the number of eosinophile cells varies as the acuteness of the attack, and hence in long-standing cases there is no increase. Calvert found that the number of these cells in the circulation bears an inverse relation to the number of embryos, they increasing in the day as the embryos disappear. In hydatid cysts of the liver the eosinophilia is considered important, it varying from 7 to 20 per cent., in one case even 40 per cent. of the total count, but not always. In the afebrile stage of malaria these cells have risen to 20.4 per cent. In dracontiasis they are reported as from 6.4 to 36.6 per cent.

VI.—**A post-febrile eosinophilia** occurs after most fevers, or at least these cells increase to the upper limits of normal. In scarlet fever during the course these cells may vary from 8 to 15 per cent., but in all other fevers during the height they are diminished, and as the temperature drops they rise; in pneumonia, *e.g.*, to 5.7 per cent., absolute number, 430; acute articular rheumatism, to 9.4 per cent., absolute number, 970; in malaria one day after the attack 20.34 per cent., or 1486; varicella, 16 per cent.; measles, 5 per cent.; rickets, 20 per cent.

VII.—During a **positive tuberculin injection** the cells fall and then rise even to 26.9 per cent. (3220). In one case reported by Grawitz their absolute number was 41,000 out of a total of 45,000 leucocytes.

VIII.—In diseases of the **genital organs** these cells are often increased; in all ovarian diseases except cancer; this was true in ten of eighteen ovarian cysts and abscesses; in gonorrhœa, especially the posterior urethritis and prostatitis, they are increased.

IX.—**In malignant disease** they sometimes constitute from 7 to 10 per cent. of the leucocytes, in one case of lymphosarcoma even reaching 60,000 cells.

X.—After **certain medicines,** as camphor (to 9 per cent.) or the

inhalation of carbon dioxide they rise, although this increase is inconstant and rare.

XI.—In **diseases of the sympathetic nervous system.**

The ORIGIN of eosinophilia does not concern the clinical microscopist. He notices that in certain smears from vesicles he finds large numbers of these cells, and that in those conditions with local accumulation the count in the blood is usually normal. There is a group of rare cases with certain cells increased in the blood concerning which it is a matter of opinion whether they are eosinophiles or neutrophiles. Were this the experience of one or two men their technique or their judgment might be exposed to criticism, but enough now have found this difficulty to justify the statement that in some cases, rare, it is true, there is a cell the characteristics of which are intermediate. Such occurs in trichinosis especially. (See page 470.)

Iodophilia.—The iodine reaction of leucocytes is tested with the following reagent: Iodine, 1 gm.; potassium iodide, 3 gms.; water, 100 cc.; gum-arabic, 5 gm.

A drop of this reagent is put on a slide, a fresh unfixed smear on a cover-glass is pressed down onto it, and the excess of stain then removed by pressure on the glass.

All the blood elements are stained a bright yellow, but in certain conditions some of the polymorphonuclear neutrophiles are found to contain granules which take a brownish-red color; some cells are diffusely stained; similarly staining substance is seen in the plasma.

The granules within the cells (the intracellular reaction) vary much in size, shape, color, number, and arrangement, or the substance may be diffusely present in the cell. The masses in the plasma (the extracellular reaction) are round or oval (from 2 to 8 microns in diameter), and often suggest masses deposited from disintegrated cells.

In judging the degree of the test, both intensity and number of cells affected are to be considered.

Why the test is given, what it means, is very uncertain. The substance present is supposed to be glycogen, but since all normal leucocytes contain this substance, it must be present here in some particular form.

The reaction is positive in the greatest variety of conditions; in pernicious anæmia, in severe secondary anæmia, but not in chlorosis, and the moderate grades of anæmia; it is always positive in leukæmia. The number of cells bears a direct relation to the acuteness of the attack, and it is said has a prognostic value. La Franca found them in chlorosis, and considered only a large number of affected cells important. Locke considers it a test independent of, but of nearly equal value with, leucocytosis. It is positive in nearly all cases of septicæmia, especially those with leucocytosis, in cases with purulent

exudates, hence especially pneumonia. It is invariably present in septic conditions due to any cause if of any severity (Locke).[57]

A clinical importance is claimed for the reaction in certain diseases, as in appendicitis. Locke found the intensity of reaction to depend on the severity and duration of the process in the appendix and the amount of septic absorption from the focus. But the point of greatest importance is, he claims, the occurrence of a marked iodine reaction without leucocytosis in some of the most virulent cases. After operation with free drainage the reaction disappears within forty-eight hours.

The pendulum has swung in regard to the value of this test, and many now condemn it as of little value surgically (Reich). Küttner gives it some prognostic value, an increase in intensity of the reaction being a bad sign.

The gynæcologists claim it is of value in diagnosis of pelvic abscess; in ovarian cyst with twisted pedicle and high leucocytosis, for instance, the test is negative; and similarly other conditions without pus formation.

Blood Platelets.—Blutplätchen, Plaques (Kemp, Osler), Hæmatoblasts (Hayem), (Plate II, 23). In the blood are the so-called "third corpuscles," small colorless bodies containing no hæmoglobin, about three microns in diameter, round, oval, or rod-shaped, according to the view-point, without a biconcavity, bluish, soft, homogeneous or granular, but not sticky and glistening when perfectly fresh as they are so soon after, which look and stain like nuclear material; they contain no nucleus, no membrane, and have in an ordinary fresh blood preparation a peculiar bluish refractivity like the protoplasm of a non-granular leucocyte. Platelets when perfectly fresh are slightly granular, but at once when removed from the blood-vessel become hyaline and glassy, then pale, and disappear, or unite to form a granular mass. Two important characteristics are to be emphasized. A platelet is soon a very sticky body, hence unless special fixing fluids are used it attaches itself at once to the glass, to other corpuscles, or platelets collect in masses of three to five normally, but of hundreds when increased much; it is very fragile, hence goes to pieces rapidly, even in a few seconds. This disintegration is particularly true of the platelets in masses, hence when they are increased, and the result is the so-called Schultze's "granular masses" (see Fig. 114), from the periphery of which radiate fibrin strands, and at the edges of which are vacuole-like areas, the so-called "viscous metamorphosis" of Eberth, or the "mucoid degeneration" of Osler.

Stained with the usual Romanowski mixtures, they are seen normally in groups of one to ten; they would seem to be composed of

[57] Jour. Med. Research, January, 1902.

nucleus and protoplasm, the nucleus consisting of rows of blue or reddish dots sometimes arranged in a spherical mass, the protoplasm-like substance sometimes hardly seen, sometimes swollen to give them almost the size of a red corpuscle.[58]

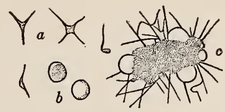


FIG. 114.—Platelets (copied from Osler): *a*, platelets in irregular shapes; *b*, with clear areas; "*c*," Schultze's granular mass.

When the platelets rest on cells they resemble malaria parasites. It is to be noted that they have not the definite structure of the parasite, that they are surrounded by a clear zone from which the hæmoglobin has been pressed out by the platelet, while the protoplasm of the corpuscle comes up exactly to the parasite.

Their size in the fresh specimens varies from 2.5 to 5 microns in diameter (Determann); 1.5 to 3.2 microns (Osler); 2 to 7 microns (Preisich and Heim). In general their size varies inversely as their number; that is, the more the platelets the smaller they are. Their fragility also is more marked when they are increased. Some soon show clear areas, either in the centre or or one side, or on the whole periphery; others become crescents, triangles, quadrangles, spindles, threads, etc. (see Fig. 114, *a*, *b*). It is much the best to study them at a temperature not over 40° F., for then these changes are much slower, requiring minutes instead of seconds.

It is customary to call wrongly anything a platelet which is smaller than a red blood-cell and does not contain hæmoglobin. The blood certainly contains a certain amount of cell detritus, but the term platelet should be reserved for bodies which have a peculiar bluish refractility, are very sticky, and soon go to pieces. Anything floating in the plasma in a blood preparation to which a fixing fluid has not been added is probably not a platelet, although it may resemble it perfectly. Buds from red cells lose their hæmoglobin, become granular or glassy, "and cannot then be told from platelets;" "inner bodies" extruded from red cells, after undergoing certain degenerative changes, "cannot be told from (degenerated) platelets." One must decide whether to call platelets all fragments which look like degenerated platelets, or to compare them with platelets studied under the best conditions; easy theoretically, but hard when one is counting them.

Specimens of the platelets are best obtained in the following manner: A drop of Picini's fluid (mercuric bichloride, 2; sodium chloride, 4; glycerin, 26; water, distilled, 226), or Hayem's fluid (water, 200 cc.; sodium chloride, 1 gm.; sodium sulphate, 5 gms.; potassium iodide solution [water, 100, potassium iodide, 5, iodine in excess], 35 cc.), or the fluid recommended by Kemp (0.9 per cent,

[58] See also Puchberger, Virchow's Arch., 1903, vol. clxxi. p. 181.

sodium chloride solution in 2.5 per cent. formalin), or Determann's fluid (distilled water, 160; glycerin, 30; sodium chloride, 1; sodium sulphate, 8; methyl violet, 0.025 parts). The best fluid, however, is a 10 per cent. sodium metaphosphate solution. A drop of the fluid is placed on the well-cleaned tip of the finger; the skin is then pricked through this drop so that the blood will at once mix with the fixing fluid; a drop is then placed on a slide and covered with a cover-glass.

To COUNT the platelets, the relation between them and the red blood-cells is counted in a fresh preparation. An ordinary red blood-count is then made, and from this the number of platelets per cubic millimetre may be easily calculated. Helber [59] counts them directly. The blood is quickly mixed with 10 per cent. sodium metaphosphate in a pipette giving a dilution of 1 : 30, and is then counted on a ruled slide similar to the ordinary counting-chamber, save that the thickness of blood-film is 0.02 mm.

The normal number per cubic millimetre has been found 250,000 (Osler); 225,000 (Determann); 245,000 (Enden). Yet the number varies much in the same person at different hours of the day, and in general the physiological variations are so considerable that only very great ones are to be considered clinically important. But Helber found no great daily variation (190,000 to 260,000; average 228,000. The remarkable closeness of these average figures is interesting). In the new-born for the first few days they are very few. In certain diseases they are very many; in other diseases very scarce. It is hard to classify these diseases, but most agree that they are increased in anæmias due to any cause, especially post-hemorrhagic, during the blood regeneration, and may be related to the red blood-cells as 1 : 10; the increase is surely a good sign; they are increased in chlorosis, decreased in pernicious anæmia and in any severe anæmia which is doing poorly. The greatest increase is in splenomyelogenous leukæmia, very low counts in lymphatic (Pratt). They are increased in chronic diseases with cachexia, and in conditions with malnutrition generally, the blood showing the marked changes of hydræmia, low specific gravity, poikilocytes, etc. The number of the platelets varies with the amount that the disease affects the blood, hence there are more in cancer and in nephritis than in the anæmia due to cardiac disease, tuberculosis, etc.

During acute fevers of long duration they are at first diminished, but increased during the third or fourth week as the patient begins to get weak. In typhoid fever a rapid diminution is considered a bad sign (Türk). In short sharp fevers there is at first a decrease, then a reactionary increase, the curve often resembling that of the leucocytes. The more acute, more severe, more threatening the disease, the higher

[59] Deutsch. Arch. f. klin. Med., 1904, Bd. 81, p. 316.

the temperature, the fewer the platelets, so that in malaria and pneumonia not one may be found. After the temperature drops, especially if by crisis, the platelets may rise above normal in twenty-four hours and continue so for two to three days, then return to normal. In erysipelas and septicæmia there is no preliminary decrease, but an increase from the start. In acute articular rheumatism there is a great increase.

Cases reported with total absence were a moribund case of pneumonia and one of nephritis, a case of pernicious anæmia and one of purpura.

Pratt [60] found no relation to exist between the coagulation time and platelet count.

The MEANING of the platelets has been much disputed. Donné considered them "globulins;" Schultze, fragments of broken-down leucocytes (also Howell); Bizzozero said they were independent corpuscles, a view which Dr. Osler also holds; Löwit said, artefacts; Hayem is the only one who considers them as very young red blood-cells. For several years the belief in these as independent corpuscles was held, until in 1897 Arnold taught that they were fragments constricted from red blood-cells or fragments of cells which had gone to pieces. Determann, following this work, pointed out that their number was usually increased in the same proportion that the red blood-cells showed signs of degeneration, that their fragility and their size depended on their number; he considered them merely a measure of the resistance of the red cells (Mosso). Schwalbe, Müller, *et al.*, consider this platelet formation from red cells a necessary preliminary step in coagulation.

Maximow [61] considers them the extruded inner body of the nucleoid. In specimens properly stained (Maximow used methylene blue and eosin, hence any of the modified Romanowski stains will do) all steps of this process can be found. Some cells have a blue-stained centre; in others this blue body projects from the cell; again it lies in a depression on the edge of the cell; others lie free. Sometimes the cell looks like a bomb bursting and discharging this mass. Engel thinks these masses are remnants of the nucleus, Maximow says not. Preisich is one of the last to insist that they are the extruded nuclei of the red blood-cells, hence are in constant process of formation; that animals with nucleated reds have no platelets; that platelets increase as the reds increase, and (this point is hardest to accept) that eosinophile leucocytes are white phagocytes which have ingested platelets.

On the other hand, platelets occur in greatest numbers where the polymorphonuclears are breaking down, as in splenomyelogenous leukæmia, and when a leucocytosis is subsiding; and least where these cells are the least numerous, as in pernicious anæmia, lymphatic leukæmia, *et al.*

The next work of importance is that of Deetjen,[62] who, using a special agar plate, considers that he has proved them independent cells, motile, and nucleated.

Deetjen's method is as follows: Five grammes of agar agar are boiled in 500 cc. of distilled water for thirty minutes to dissolve it, then filtered through a folded filter. To each 100 cc. of filtrate are added 0.6 gm. of sodium chloride, 6 to 8 cc. of 10 per cent. sodium metaphosphate ($NaPO_3$), and 5 cc. of 10 per cent. acid potassium phosphate (K_2HPO_4). After adding these salts the fluid shouid not be boiled nor much heated, lest the metaphosphate be converted to orthophosphate. The solution should now be clear. A drop is placed on a slide and allowed to cool.

[60] Jour. of Med. Research, August, 1903.
[61] Arch. f. Anatomie u. Physiologie, Anat., Abth. 1899.
[62] Virch. Arch., May, 1901, vol. clxii.

The most of the agar surface is cut away, leaving a smooth area about 2 mm. square. On this the blood-drop is placed and at once covered by a cover-glass.

The specimens may be fixed by allowing 1 per cent. osmic acid to run under the slip; the cover is then raised, washed in water, then in 96 per cent. alcohol for one minute, then stained in hæmatoxylin and eosin.

Deetjen believes them to be actively amœboid, the amœboid motion requiring the above salts for its best demonstration. In such agar specimens they are round or elliptical disks, then in one or two minutes show a more refractive round inner body with a greenish tinge and a periphery of pale protoplasm which is "in active motion," changing shape so rapidly, and position as well, that it is hard to draw them. This may continue for hours; best on a warm stage. The stained specimens show the protoplasm and the inner body which takes a nuclear stain, and seems to consist of a chromatin net-work. The ease with which this can be seen depends on the amount they have "spread out." Some are larger than red blood-cells.

Wlassow, who added dilute mercuric chloride solution to fresh blood and could see the platelets "leave the red cells," was an especially severe critic of Deetjen's work, and pointed out that although platelets do change their shape they never resume their original one; they may extrude "pseudopods," but they never show true amœboid motion; chloroform will stop the amœboid motion of leucocytes, but not that of platelets; their nucleated structure is not proved; amœboid motion is never seen in platelets in the circulation; Deetjen uses an agar field, which would much favor the production of diffusion currents; Wlassow, therefore, still believes them to arise in the red cells. Wlassow made quickly a fresh specimen of blood, then ran under the cover-glass a drop of one-fifth concentrated mercuric chloride solution; at once the reds become granular and a small refractive hyaline area appears, usually at the periphery. From this a bud develops which is sometimes an irregular mass of granules which increases and may become more thorny, and then separates from the corpuscle. This body may or may not contain hæmoglobin, those which do later lose their color and then cannot be distinguished from platelets. Others have confirmed Deetjen's work (Dekhuysen and Kopsch).

We have observed most of the above phenomena, but have seen no true amœboid motion, that is, a change in position which the changes in shape will explain. All look more like mechanical and chemical processes.

Kemp, in his recent interesting work on the blood at a high altitude, is confident some platelets do contain hæmoglobin, and hence is in doubt as to their origin; formerly he had been a firm believer in them as third corpuscles.

Reaction of the Blood.—To litmus the blood is alkaline, and various methods have been proposed to determine the amount of this alkalinity. If by alkalinity is meant a preponderance of free OH-ions in the blood, the physical chemists have taught us that the blood is an almost neutral fluid, quite so when defibrinated, slightly alkaline when arterial, while the serum is almost as neutral as distilled water. But by alkalinity the clinical chemist means the acid-combining property of this fluid, or the amount of alkali in it which can be substituted by an acid. This is of interest and of considerable importance.

The reaction of the blood is due to the alkaline phosphates of sodium and the alkaline earths, and to the alkaloidal bases (Labbé). Yet chemically the blood acts as an acid from the presence of acid salts; that is, it contains unstable acid salts which react to color indicators as feeble bases, but which behave as acids, since, in the presence of true alkali, they become neutral. Brandenburg divides the alkaline components into two parts. The first is the diffusible alkali, the native,

or the mineral alkali, that is, the bases which are bound to carbon dioxide. This is diffusible, and may be measured by bringing the blood into contact through a diffusion membrane with alkaline fluids of various concentration until one is found which in contact with blood does not change its alkalinity. The other, and greater in amount, is the alkali- and acid-binding value of the proteids which varies directly with the amount of albumin, hence chiefly with the blood-count. The diffusible alkali is to the total as 1 : 5 in the case of the total normal blood; in the serum, as 1 : 2; in the corpuscles, as 1 : 8; and in cases of anæmia of various grades that of the total blood may vary from 1 : 3 to 6.

The alkaline tension (that is, the diffusible alkali) is rather constant, about 60 mg. of NaOH per 100 cc. of blood. The total alkalinity depends much on the blood-count, and hence is subject to great variations. The alkaline tension and the molecular concentration of the plasma run almost parallel.

The alkaline tension has been found reduced in diabetes mellitus, in uræmic coma, in pneumonia, and in certain cases of nephritis (small contracted kidney and acute toxic nephritis). These cases, then, may be said to show "acidosis," or evidence of acid intoxication.

The alkalinity of the blood can be varied for a short time by alkaline drugs, and alkaline rectal enemata, but the effect is very temporary.

Determination of the Alkalinity.—There is hardly a determination which the clinician would more gladly make than the alkalinity of the blood. It would be of such importance in diagnosis, in prognosis, and in therapy; and yet the methods are notoriously inexact.

The reaction of the blood to litmus has little absolute value, since it does not show the carbon dioxide the chief acid of the blood, and yet this is the indicator which has been commonly used, and the results obtained have a certain empirical value.

(1) Landois-v. Jaksch Method.—By this method it is attempted to determine the alkalinity of the plasma by adding to the blood an acid solution of Glauber's salt of such composition that the blood is not laked. Eighteen mixtures of this solution are used.

Solution 1: 1 cc. contains 0.9 hundredth-normal tartaric acid and 0.1 cc. conc. Glauber's salt solution.

Solution 2: 1 cc. contains 0.8 hundredth-normal tartaric acid and 0.2 cc. conc. Glauber's salt solution.

Solution 3: 1 cc. contains 0.7 hundredth-normal tartaric acid and 0.3 cc. conc. Glauber's salt solution.

Solutions 4 to 9: this series continued.

Solution 10: contains 0.9 thousandth-normal tartaric acid and 0.1 cc. conc. Glauber's salt solution, etc.

Solution 1 equals 360 mg. NaOH in 100 cc. of blood. Of these various fluids, 1 cc. is measured into small receptacles, and 0.1 cc. of blood, accurately measured, added, mixed, and the reaction tested at once, that is, in less than one and a half minutes, with litmus paper. V. Jaksch, who has used this method extensively,

considers that in 100 cc. of blood is alkali equal to 0.26 to 0.30 gm. NaOH. This method, although valuable results have been obtained with it, is unsatisfactory since the error from disregarding the corpuscles is great.

Tenth-normal tartaric acid contains 7.5 gms. of the pure acid per litre of water. From this the other strengths are easily made.

Löwy Method.—This is still the best method for the total alkalinity of the blood. In a special flask, on the neck of which are marks indicating 45 and 50 cc., are measured 45 cc. of 0.25 ammonium oxalate. To this are added 5 cc. of blood removed by hypodermic syringe from a vein of the forearm. The blood is therefore laked and will not coagulate, hence the alkalinity determined is that of the total blood. This fluid is then titrated with twenty-fifth-normal tartaric acid, lacmoid paper saturated with magnesium sulphate as indicator. By this method 100 cc. of blood contain from 400 to 600 mg. of sodium hydroxide (Strauss, 300 to 350).

Lacmoid is a product of resorcin, hence is allied to litmus. It is prepared by heating gradually to 110° C. a mixture of 100 parts of resorcin, 5 of sodium nitrite, and 5 of water; after the violent reaction subsides it is heated to 120° C. until evolution of ammonia ceases. The residue is dissolved in warm water and the lacmoid precipitated by hydrochloric acid. The precipitate should be dissolved and reprecipitated several times to purify it, then washed free from acid and dried. It is then dissolved, 3 gms. per litre, in dilute alcohol. The paper is dipped in this solution, then dried.

With litmus Orlowski found the alkalinity to be 240 to 267 mg. NaOH; with lacmoid 269 to 289 mg. (Engel method).

The Engel apparatus is based on the Löwy principle, but requires a much smaller amount of blood, not much more than for a blood-count. A similar blood mixing pipette is used in which the blood is measured and laked, then titrated as above.

Dare's Method.—Dare has introduced an entirely new method. To avoid color-tests, which are confused by the hæmoglobin, he determines the point at which the absorption bands of the spectrum of oxyhæmoglobin disappear in a mixture of blood to which amounts of tartaric acid are successively added. The apparatus consists of a hand spectroscope, a pipette which measures 20 cmm. of blood, and a graduated test-tube. In the test-tube are placed a few drops of water, into which the blood is washed from the pipette with distilled water and more is added until the well-mixed diluted blood just reaches the zero point. The two-hundredth-normal tartaric acid solution is then added.

Tartaric acid (pure), 0.075 gm. dissolved in distilled water; alcohol (95 per cent.), 20 cc.; distilled water, to 200 cc., is the formula given. The chemist, for the sake of accuracy, will make it up in much stronger solution, and then dilute to this proportion.

Each addition is well mixed and the presence of the oxyhæmoglobin lines determined by the hand spectroscope. An artificial light at a constant distance is recommended. The addition of acid is continued until these two lines disappear. A direct reading of the number of milligrams of sodium hydrate may then be made. Dare gives a list of cases in which the alkalinity was decreased and of others in which it was increased.

The apparatus we find ingenious and very constant in its results, and hope to gain some practical value from its use.

Determination of Carbon Dioxide.—This method, which at first glance seems the most valuable, since one of the chief functions of the alkalinity is to bind the carbon dioxide, is both hard in technique and not at all perfect in theory. In diabetic coma the amount of gas has been found diminished to from one-half to one-third the normal amount. It has also been diminished in fevers, cancer, and leukæmia. But variations in the gas are not necessarily the same as variations in alkalinity.

Rigler's Method.—Rigler's method is simple, and has found several champions. Into a weighed flask containing 10 cc. of absolute alcohol is poured a small amount (3 or 4 cc.) of blood and is again weighed. Thus can the amount be better determined than volumetrically. This is allowed to stand for about half an hour; 10 cc. of distilled water are then added and it again allowed to stand half an hour. It is then titrated with fiftieth-normal H_2SO_4, using litmus as indicator.

Brandenburg's Method.—The total alkalinity is first determined by a modified Löwy method, lacmoid as indicator. Three portions of defibrinated blood of at least 20 cc. each are placed in vessels for osmosis work, each separated by diffusion membranes from NaOH solutions in 0.8 per cent. NaCl, the one of which is one-fourth, the second one-fifth, the third one-sixth the alkalinity of the total blood. At the end of twenty-four hours the alkalinity of the fluid without the membrane, and of the blood-mixture within, are determined. Limits between which the diffusible alkalinity stands are thus determined.

It will be seen that this has limited clinical value as a routine determination, but has taught much concerning our other methods and results.

Salkowski's Method.—Salkowski's method is certainly simple. A known amount of ammonium sulphate is added to the blood and the ammonia of the salt thus set free determined by Schlösing's method.

Under a bell-jar are placed 20 gms. of finely pulverized ammonium sulphate which have been dissolved in 20 cc. of water. In a receptacle above this are placed 10 cc. of fourth-normal sulphuric acid. One then pours into the lower dish 10 cc. of blood. The measuring-glass to be used for the blood is first washed in 1 per cent. sodium oxalate solution to prevent coagulation. The blood is mixed with the ammonium sulphate solution, and the whole covered at once with the bell-jar. In five or six days all of the ammonia set free from the ammonium sulphate will have been taken up by the sulphuric acid and its amount determined by titrating this. By this method the alkalinity has been found for men, 350 to 400 mg. of NaOH per 100 cc.; for women, 300 to 350 mg.

In fever cases and anæmia cases it is diminished.

The conclusions from these results are rather hard to state, the different methods giving quite different figures (Bezançon and Labbé):

Rumpf, Landois: 182 to 218 mg. NaOH per 100 cc. of blood.

Lépine: 203 to 276 mg. NaOH per 100 cc. of blood.

Bererd: 450 to 500 mg. NaOH per 100 cc. of blood.

Tauszk: 700 to 800 mg. NaOH per 100 cc. of blood.

Brandenburg: 300 total, 60 diffusible per 100 cc. of blood.

Each method, however, has a certain empirical value. It has been found that the alkalinity in the case of men is greater than in women and is still greater in children. At birth high, it reaches a minimum in from one to three years, reaches the point normal for adult life at about sixteen, and slightly diminishes in old age. The daily variations in normal persons are slight. During digestion the alkalinity is slightly increased (due to the secretion of acid in the gastric juice). It is decreased by excessive exercise, supposed to be due to the formation of lactic acid. As a measure of the effect of various therapeutic procedures no satisfactory results have been obtained, the effect of various alkalies or acids being very slight and transitory. The blood seems usually well able to resist any change in its reaction.

The alkalinity of the total blood is always greater than that of the serum, since the corpuscles play no small part; the alkalinity rapidly diminishes until coagulation; then is almost constant. Zunz found that in two minutes it fell from 330 to 170 mg. per 100 cc. The arterial blood is slightly more alkaline than the venous blood, but the difference is not great.

Pathologically it is diminished in severe anæmia, especially the pernicious, but not in chlorosis; in the high fevers, diphtheria, scarlet fever, and measles, increasing even to above normal during convalescence; in uræmia, osteomalacia, and just before death. The most interesting cases are the acidosis due to cancer, malnutrition, profound cachexia, and above all else the coma of diabetes mellitus, in one of which cases the amount of acid per kilo was equivalent to 1.75 grammes of HCl.

Animal experimentation by Fedor, Rigler, and others showed that animals infected with pathogenic organisms first respond with an increase in the alkalinity, which is followed by a decrease. If the infection be fatal the decrease continues until death; if the animal recovers, the alkalinity will then rise until above normal. There seems, therefore, to be a causal relation between the reaction of the body to these pathogenic organisms and its alkalinity, the resistance being better the higher the alkalinity. In the case of diphtheria a dose of antitoxin will cause a rise, which begins in about two hours, reaches a maximum in from ten to twelve hours, and drops to normal on the third day. Rigler, in a long series of experiments on animals, found that a diminution in the alkalinity followed every time the inoculation. This was most marked in the fatal cases, but was very low in most chronic, not necessarily the most virulent cases. During convalescence there was always a rise. This reaction is not specific.

Already the ammonia of the urine has proved itself of practical value. We hope soon that the alkalinity of the blood upon which the reaction of the urine certainly depends will be as easily and accurately determined, for the clinical observations seem to indicate that it would be very valuable. As yet it is practically useless.

Urea in the Blood.—A very simple method given by Herter [63] is as follows: A carefully measured quantity of blood is treated with three to four times its volume of absolute alcohol and allowed to stand twenty-four hours. The filtrate and washings are evaporated to dryness at moderate temperature, the residue taken up again in absolute alcohol, and again filtered. The filtrate and washings are again evaporated to dryness, and the residue dissolved in a little water. The nitrogen of this solution is determined by the sodium hypobromite method, exactly as in the urine. Other nitrogenous extractives of the blood, as creatin and lecithin, will also furnish a little nitrogen, but these are small in amount and are not wholly broken up, while urea is by far the most abundant. This method is sufficiently accurate for clinical work.

Anæmia.—Grawitz defines anæmia as a deterioration of the blood qualitatively and quantitatively as regards one or all of its constituents —the plasma, the corpuscles, and the hæmoglobin. The term as generally used means a reduction per cubic millimetre of the red blood-cells either in number or in volume. But these cells are really a relatively unimportant part of the total blood, having, so far as we know, but one function, and that a relatively minor although indispensable one, of carrying oxygen to the tissues, while the plasma contains the constituents upon which depend the life and health of the body, including the red corpuscles. In the plasma are the raw materials from which each cell gets new material for its structure, the food and fuel which it may use, and the many and as yet unknown bodies for its defence.

By anæmia, properly speaking, we should mean a diminution in the total volume of undiluted blood. The proof of this is impossible. The reduction in volume does occur, as observations at the autopsy table show; in cases of emaciation, for instance, and with a practically normal blood-count. On the other hand, by dilution with tissue-lymph the blood after a hemorrhage will maintain its volume, while the dilution is manifested by the red blood-count. Lastly, in other cases, as of pernicious anæmia, there is evident diminution in volume of blood and of the corpuscles.

But the essence of an anæmia is a poverty in the plasma of the protoplasm-building bodies, that is, of the proteids. Some have considered that this was best determined by measuring the amount of water in the plasma, which may be supposed to increase as they decrease, but this has proved unsatisfactory since a dilute plasma may mean only an increased volume, while in the severest anæmias (clinically) with low count the plasma can protect itself remarkably well, and chemically be almost normal.

[63] Jour. of Exp. Med., vol. iv. p. 119.

From the practical point of view a diminution in the percentage of hæmoglobin is accepted as the most sensitive index we have of any deterioration of the blood, and a diminished red blood-cell count a sign of a slightly more advanced grade, but first in value.

By *oligocythæmia* or *hypocythæmia* is meant a relative diminution in the number of red blood-cells; that is, those of a unit volume of blood are absolutely diminished. This may be due either to an actual reduction in number of the cells in the body, or to an increased amount of plasma. By *oligochromæmia* is mean a diminution in the amount of hæmoglobin per unit volume of blood. By *color-index* is meant the percentage of hæmoglobin divided by the percentage of the red blood-cells, 5,000,000 cells considered as 100 per cent. (See page 466.)

By *oligæmia* is meant a diminished amount of blood in the body. This may be suspected, but cannot be proved. *Oligæmia serosa* is an oligæmia of diluted blood; *oligæmia sicca,* an oligæmia with blood qualitatively normal. *Hydræmia* means an increased percentage of water, and occurs whenever there is a diminished amount of albumin. *Polyplasmia* is an increase in the volume of the plasma, supposed to occur in chlorosis; *oligoplasma,* a decrease, which occurs in certain cardiac diseases. By *plethora vera,* an increase in the total volume of blood. This can only be suspected.

By the *hæmatopoietic organs* one usually means the organs forming red and white corpuscles; the bone-marrow, spleen, and lymph-glands. The bone-marrow certainly is the building-place for red corpuscles and many leucocytes; the spleen is active, perhaps, after a severe hemorrhage, but otherwise is probably unimportant so far as the red blood-cells are concerned; it is possibly important in the formation of leucocytes, and is quite probably the organ which removes the old cells; the function of the lymph-glands as hæmatopoietic organs is still in doubt.

The red blood-cells have, so far as we yet know, the one function of transporting the oxygen to the tissues, an indispensable but relatively minor function of the blood. The function of the leucocytes is still imperfectly known; in general, they are said to be important in immunity, in the protection of the body against invading organisms, or toxins; in nutrition, since by means of them a great deal of neutral fat is absorbed into the intestine; and by going to pieces they certainly raise the albumen content of the blood. The function of the platelets apart from coagulation is not well known. While these functions of the formed elements are very important, those of the plasma of the blood are far more so, and we study the former because they are as yet the only index of the condition of the latter. Yet in studying the causes of anæmia those organs which form the plasma must be considered the most important hæmatopoietic organs. These are especially the intestine, the liver, and the kidneys, although every organ modifies to some degree the composition of the plasma. It is in the intestinal wall that the plasma obtains its proteid content; in the liver that the excess of carbohydrate is removed, or more furnished when necessary, that the ashes of the body are transformed to urea, etc.; and it is in the kidneys that the most of the ashes are removed. Certain of the glands with internal secretions are also important in modifying the constitution of the blood. The pancreas furnishes the internal secretion necessary in sugar metabolism, while the importance of the thyroid and the adrenal is well known. Lastly, in the muscles themselves the blood is modified, they taking up certain food constituents and fuel, and giving in return the ashes of

these bodies. It is thus seen that every organ of the body is a blood-building or blood-modifying organ, either adding to or subtracting from the plasma certain bodies, and that the blood will suffer from disease of any one of these organs but especially of the intestine, liver, and kidneys. In nearly all disease of the blood it is probably the plasma that suffers first, and in many of the anæmias the diminution either in amount or in value of the red blood-cells may be merely the expression of the malnutrition resulting from an abnormal plasma of the organs forming them. The corpuscles cannot stay normal long in an hydræmic plasma. In pernicious anæmia and other toxæmias the element of blood destruction is of course also very important.

The anæmias may be classified as *primary* and *secondary*. By primary is meant one for which an adequate cause cannot be assigned. Here are included chlorosis, the essential idiopathic anæmias, the simple primary and the pernicious, leukæmia, and pseudoleukæmia. By secondary anæmia is meant one for which the cause assigned seems adequate to explain the blood condition. Under these causes are grouped hemorrhage, blood poisons, malnutrition, increased albumin destruction, cachexia, and poor hygienic conditions. The above classification is purely clinical, not hæmatological, and the autopsy table not infrequently shows to be secondary an anæmia which was during life supposed to be primary. The blood pictures are by no means sufficiently distinct. Points of differential diagnosis are easy enough to tabulate, but rather hard to apply in an individual case unless marked, and yet the anæmias of known cause seldom resemble the pernicious variety and it is seldom one finds a case with the picture of secondary anæmia and the cause not known. With the clinical history, the physical examination, and the blood examination, the diagnosis is usually satisfactory, but not always.

By *hypoplastic anæmia* is meant one due to insufficient blood formation. By *consumptive anæmia* one due to increased blood destruction.

From the hæmatological point of view one separates the *chlorotic* and *pernicious* forms. These terms are used carelessly. By the former is meant a slight or no reduction in the number of red cells and a definite often considerable reduction of the hæmoglobin. In this list are nearly all the milder grades of secondary anæmias, the primary chlorosis and the leukæmias. By "pernicious" in this connection is meant a great reduction in both cells and hæmoglobin, equal or with the color-index higher than I. Such cases are the primary pernicious and rare cases of almost any extreme form of secondary anæmia.

Secondary Anæmia.—A secondary anæmia is, from the point of view of the pathologist, one of which the cause, known or suspected, seems sufficient to explain the condition. But whatever the cause, the picture which the term brings to mind is that of a blood of which the hæmoglobin is more reduced than the count of reds, and the plasma hydræmic. The red cells are usually smaller, of lighter weight, while some are large and "waterlogged."

Cabot suggests a classification for the secondary anæmias which is useful. *Mild cases* are those with a normal count, but with the hæmoglobin diminished; specific gravity slightly lowered; the count of the red blood-cells normal, yet a good many of them of light-weight, *i.e.*, small and pale in appearance, are seen. A *moderate grade* is one with a normal count, but the reds show qualitative changes; degenerations, microcytes, poikilocytes, crenated cells; the cells stain abnormally; there is less tendency to rouleaux formation. *Severe cases* are those with both qualitative and quantitative changes; the count, however, is not much reduced except in the anæmias of childhood, after large hemorrhages, in malaria, and in acute septicæmia. *Very severe cases* are those with all of the above mentioned changes, and in addition evidences of degeneration and destruction of the cells; evidence of regeneration (nucleated reds) will also be present.

Blood Picture.—In secondary anæmia the blood may grossly be pale. The reds are less reduced than the hæmoglobin, and their *count* may be normal. In severe cases, however, there will be a great reduction; in v. Limbeck's for instance with recovery, 306,000. A reduction of 1,000,000 cells, Bezançon and Labbé consider a mild hypocythæmia, one of from 2,000,000 to 3,000,000, an intense, while if the cells are reduced to one million, an extreme hypocythæmia.

The reduction in *hæmoglobin* is the constant and most important feature, and the best index of the grade (yet see page 466). The color-index is lowest in cases due to cancer, hemorrhage, and gangrenous processes, yet in none of these cases is it quite so low as in chlorosis. On the other hand in cases with extreme oligocythæmia the body, if given sufficient time, seems to protect itself by increasing the color-index, that is, by the production of cells which in size or weight are normal or above normal. Some think that the high color-index of pernicious anæmia is itself not characteristic of the disease, but an expression of the low count, the body, because of the chronicity of the disease, having had time to thus protect itself, while in those cases of secondary anæmia with low count and low color-index the acute course prevents this protective measure. The specific gravity of the blood is low. The dried residue is reduced. This is especially true in the cancer cases. (In one case of cancer of the stomach with a count of 1,400,000, 15 per cent. Hb, the dried residue was only 9 per cent.)

Morphologically, the stained cells show a lack of hæmoglobin, and yet a good many are normal. In many the biconcavity is very evident, and pessary forms are common. The polychromatophilic degeneration is common, is seen within twenty-four hours after a hemorrhage, but bears no relation to the hæmoglobin-content of the cell. The number of these basophilic cells runs fairly parallel to the grade of the anæmia,

so much so that this easy method has been suggested as a substitute for the more difficult of blood-counting (Walker).

Poikilocytes occur only in the severest cases, unless the term be used to include the variations in size, for the cells are much reduced, although unevenly so. Microcytes always occur, some even 2 microns in diameter. Large cells with "acute dropsy" have been described.

Nucleated reds vary much in number. This bears no relation to the anæmia, either its grade or its cause. They are abundant in some cases, as in acute post-hemorrhagic anæmia and in some chronic cases, while in others of the same degree they are absent. They may occur in crises (see page 479). The cells are normoblasts as a rule, microblasts occur in the severe post-hemorrhagic type, and megaloblasts are exceedingly rare except in cases of malaria and other diseases which affect the bone-marrow.

The *leucocytes* vary, depending on the cause of the anæmia and its complications, from a leucopenia to a leukæmic condition. Anæmia is a great stimulus to the bone-marrow, which during convalescence increases the count of the white blood-cells as well as of the red, hence, as a rule, there is a moderate leucocytosis with an increase in the polymorphonuclear neutrophiles. In the other cases it is very hard to explain the picture. The *eosinophiles* vary much, from few to an extreme eosinophilia. As a rule they are at the upper limits of normal, and if further increased some other reason than the simple anæmia is the cause.

The *platelets* are increased, even doubled in number. This is always true in the post-hemorrhagic cases.

Acute Post-Hemorrhagic Anæmia.—This anæmia may be acute or chronic. The loss of one-half to two-thirds the volume of blood at one time is fatal. Women tolerate hemorrhage better than men, and children least well of all.

The character of this anæmia depends upon the hemorrhage; whether the loss of blood occurred all at one time or at intervals. The clinical picture of these forms is very different.

The blood immediately after a hemorrhage is normal qualitatively, then, as the tissue-lymph is poured in to restore the volume, the count and the hæmoglobin diminish and the specific gravity becomes somewhat less since the lymph is richer in water than is the plasma. The color-index should remain "1" for a short time, then decrease, since the new cells hastily formed are "light weight," smaller in size, paler in color, and more easily degenerated, both as regards their shape and staining qualities. It is possible, of course, that some of these degenerated cells are old cells allowed to remain in the circulation longer than is normal, and that others are cells injured by the abnormal plasma.

The loss of from 50 to 70 cc. of blood even will cause an appreciable increase in water. The count falls steadily until the dilution is complete, and then as the new cells appear the count slowly returns to normal, the hæmoglobin somewhat later, it being weeks before the imperfect cells are entirely removed from the blood. The platelets are increased. The maximum hydræmia after one hemorrhage and the minimum color-index are on about the ninth day. There is often a post-hemorrhagic leucocytosis. The regeneration of the red blood-cells is rapid at first, and then slower. This early rapid increase some think due to division of the red blood-cells of the circulation, and in favor of this is the number of the small cells and of poikilocytes which are so soon found.

Nucleated reds may appear sometimes in large numbers, and disappear in about one week. They are chiefly normoblasts. Regeneration sometimes progresses in " steps" with blood crises (see page 479). The number of nucleated reds is often related more to the acuteness of the hemorrhage than to its severity.

In one case 13.7 per cent. myelocytes were found, which disappeared in three days. In another case of very severe post-hemorrhagic anæmia the polymorphonuclears were free from granules.

An early feature in the regeneration is the production of many large cells, in some cases these being the chief element of the blood picture.

Time for Regeneration after One Hemorrhage.—The table given by v. Limbeck is:

Blood loss of 4.5 per cent. of body weight, thirty days to restore loss.

Blood loss of 4 per cent. of body weight, twenty days to restore loss.

Blood loss of 3 per cent. of body weight, ten days to restore loss.

Blood loss of 2 per cent. of body weight, eight days to restore loss.

But this varies with the age, nutritional condition, diet, and therapeutic measures. Grawitz says a loss of 3 to 4 per cent. of body weight requires fourteen to thirty days; of 1 to 3 per cent., five to fourteen days; a slight loss, two to five days.

Regeneration is quickest in men between twenty and forty years of age; slower in women, and slowest in children. After the regeneration is complete there may be even a hypercythæmia. Mikulicz stated that it was unwise to operate in cases in which the hæmoglobin was already or probably would be 30 per cent. after the operation. In our cases, however, we have operated with the hæmoglobin lower with good results.

In animals regeneration may be almost entirely prevented by feeding an iron-poor diet, especially if by previous hemorrhages the iron reserve supply of the body has been exhausted—a very suggestive point in human pathology.

In a case with repeated hemorrhage following abortion (due to a drug, there was no evidence of septicæmia) the count on admission was 1,108,000; hæmoglobin, 18 per cent.; leucocytes, 4625; temperature normal.

In a case of hemorrhage from a badly crushed arm and after infusion the red cells fell in thirty-six hours from 5,000,000 to 3,000,000, the hæmoglobin from 70 to 50 per cent. In a case of metrorrhagia the hæmoglobin fell to 19 per cent., yet the patient recovered; another with two post-partum hemorrhages showed two weeks later 11 per cent. hæmoglobin, yet recovery.

Among the causes of this acute anæmia are traumatic hemorrhage, tubal pregnancy, in which a rapid anæmia is a bad sign, abortion (see above), uterine submucous tumors, ulcers of duodenum and stomach, typhoid ulcers, phthisis, aneurisms, varicose veins of œsophagus, rectum, or legs, the hemorrhagic diatheses, and hemorrhagic pancreatitis.

A case of *purpura hæmorrhagica* of eight weeks' duration[64] was admitted with a count of 696,000; hæmoglobin, 17 per cent.; leucocytes, 4000 (small mononuclears, 75 per cent.). At death seven days later the red count was 483,000, no poikilocytosis (since too acute?), no nucleated reds, no eosinophiles.

Ewing mentions a case of three weeks' duration with repeated epistaxis and a red count of 456,000.

Anæmia from Chronic Hemorrhage.—The conditions which obtain here are very different from those following acute hemorrhage. By chronic hemorrhage is meant a succession of hemorrhages at such intervals that the patient cannot recover from the one before the next loss of blood occurs. If the intervals are long enough for complete regeneration the conditions are merely those of acute hemorrhage, and the amount of blood lost in the aggregate may be enormous, as was well seen in the former days when venesection was a common practice. Ehrlich mentions a Russian physician with pulmonary tuberculosis, who in six and a half months lost twenty kilos of blood—that is, four times the total amount,—and yet recovery was perfect. In case the intervals are shorter, even though the total amount of blood lost be relatively small, the results are more serious. This is well seen in the repeated small hemorrhages from the nose. A case with repeated epistaxis due to telangiectasis of the nasal mucosa was admitted here several times, once with red cells, 2,288,000; hæmoglobin, 18 per cent.; leucocytes, 2800. Scurvy, especially if with much hemorrhage, causes a secondary anæmia sometimes of severe grade (370,000), but usually moderate (reds averaging from 3,000,000 to 4,000,000). There is often a leucocytosis due to some complication, otherwise leucopenia. In one case of this clinic the red cells were 2,200,000; hæmoglobin, 40

[64] Billings, Johns Hopkins Hosp. Bull., May, 1894.

per cent.; leucocytes, 2850. Other cases follow hemorrhage from lungs, uterus, hemorrhoids, from intestinal ulcers, cancer of the stomach, intestinal parasites, cancers, etc. The severe anæmia from high and hidden piles is now attracting much attention.[65] After long anæmia the blood-building organs seem to lose their ability to regenerate the blood, and the picture becomes that of a primary anæmia, rapidly fatal and without any sign of regeneration. It is perhaps the poor nutrition of the blood-building organs resulting from the anæmia which results in the pathological direction of their activity or their entire loss of function. In other cases it is merely the result of the chronic disease causing the hemorrhage. It, therefore, takes much longer for the blood to regenerate; in one case of hemorrhoids with a count of 2,600,000 it required eight months to reach normal (Ehrlich).

In this form of anæmia the hydræmia is considerable, the specific gravity is low, the dried residue considerably diminished. The red count is much diminished, even to 1,000,000 cells. The new reds are small and pale, and the index low, 0.5 or even 0.44. The nucleated reds are scanty, the platelets increased. Sometimes, but seldom, the picture is that of a pernicious anæmia, but the patients usually die before this picture is present, and the index progressively lowers until death, although fluctuations often enough occur. The leucocytes are increased at first while the reaction to the hemorrhage is active, and then with the pernicious anæmia leucopenia results. It seems that the loss of albumin is the most important element, and that upon which all other features depend.

Blood Poisons.—These may cause anæmia by shortening the life period of the individual corpuscles. But since there is normally a good recuperative power, the poison must be severe, or if slight continue for a long time, to have a marked result. Such poisons are produced in many infectious diseases, especially the septicæmias, scarlet fever and lues; chronic poisons, as lead, arsenic, and mercury; the toxines of intestinal parasites, as Bothriocephalus latus; those arising in the intestine as the result of decomposition of the intestinal contents and in constipation; the toxine of malignant tumors; all these cause anæmia.

The effect of these poisons is sometimes seen in the red blood-cells in the circulation, the various degenerations, and the hæmoglobinæmia (plasmolysis). Other toxines are thought not to injure the cells in the circulation, but to cause an increased activity on the part of the blood-destroying organs, the liver, the spleen, and the marrow, without any hæmoglobinæmia. One of the best illustrations of the effects of such a supposed toxine is hæmatochromatosis, with the deposition of so much iron-containing pigment. The probability is that there has been a chemical (plasmotropic) change in the protoplasm of the cells which singles them out thus for destruction. Some poisons are thought to be purely plasmotropic, as for instance, lead, the toxine of cancer, of certain bacteria, and of ptomaines. Others

[65] See Herrick, Jour. Am. Med. Assoc., September, 1902.

in small doses are plasmotropic, in larger doses plasmolytic; in other cases the anæmia is thought to depend on the great differences in resistance of the reds (Grawitz).

Anæmia of Inanition; the Anæmia of the Poor.—This form is considered by some as a simple primary anæmia, by others as a secondary anæmia but due to a variety of concurring factors the relative importance of which cannot be apportioned, such as poor food, lack of sunlight, bad air, worry, and overwork.

Starvation alone will not cause anæmia; that is, not qualitative changes in the blood, but animal experiments as well as clinical observations have shown that there is a true anæmia, that is a diminution in the total volume of blood which runs parallel to the loss of weight. The blood picture of anæmia begins with the regeneration, since with the improvement in condition the blood does not keep pace with the gain of the other organs, and hence is diluted. The blood of Cetti, who fasted ten days, showed a rise in the red blood-cells of one million, a slight fall in the hæmoglobin, while the leucocytes fell from 12,000 to 4200. Others (Grawitz) consider that in some cases there is not simple atrophy of the total blood, but a loss of albumin of the plasma, hence a true anæmia. This is more evident if the days of fasting are alternated with days of slight nourishment, since the partial restoration of volume graphically becomes apparent. This is well seen in typhoid fever during the fourth week, in which case there is a rapid fall in the blood-count.

Poor food is an important cause of chronic anæmia of the purely hypoplastic form (Immermann). This anæmia is of the purest type, since it is due to insufficiency of blood formation. It is not so much the quantity as the quality of the food which is of importance, and unfortunately for the poor the most important foods, those containing iron, are the most expensive. These cases are met with particularly in those European countries where the diet of the poor consists of bread, potatoes, and other cheap food of similar nature. In this country, where there is by no means such a large class of poor on such miserable diet, the trouble is not so much the quality of the food as its preparation, good meat and vegetables being rendered indigestible by preparation in the frying-pan. In addition to this must be included the hurry in eating and the insufficient mastication of the food, which is a common sin in all grades of society. Bunge's experiments have shown that a diet poor in iron causes anæmia in a growing child, and yet it cannot be the lack of iron alone, since even the poorest foods have sufficient of this metal to replace the actual loss on the part of the body. With a truly non-proteid diet the effect on the blood can be demonstrated at the end of six or eight days, the first effect being a slight hydræmia, later the changes in the red blood-cells, which are probably secondary to the former.

Those living in dark houses are very apt to be anæmic. This is not due alone to the LACK OF SUNLIGHT, since hæmoglobin is not exactly comparable to chlorophyll, as the illustrations given by Ehrlich show; the horses which for from ten to twenty-four years are kept at the bottom of mines in Germany without seeing sunlight have normal blood; the members of Nansen's Polar Expedition remained for one hundred and forty to one hundred and fifty days without sunlight, and yet were healthy since the other causes of anæmia were eliminated. Although sunlight may not be so important for the adult, yet it has been shown to be important for the growing organism (Schönenberger).

To live constantly in an atmosphere of BAD AIR also seems to predispose one to anæmia and an excess of carbon dioxide is cited as the real cause, and yet the real relation of this single factor to anæmia it is difficult to determine.

In review it may be said that these factors all combine to cause the anæmia of the poor, and yet of them all overwork and worry, with their serious influence upon digestion and the nervous system, are probably the most important; hence it is that "anæmia of the poor" is really a misnomer, for the rich also suffer from disturbances of the gastro-intestinal tract which render their food almost as little nourishing, and worry is perhaps more their lot than that of the poor; hence it is that anæmia is perhaps quite as common among them.

There is a group of cases we diagnosed as secondary anæmia for which no one cause can be assigned. The great majority were women; the red cells showed a mean of about 3,000,000 (2,100,000 to 3,900,000); hæmoglobin, 30 to 50 per cent.; leucocytes, about normal. Such cases improve rapidly in the ward.

As has been said, one of the most important hæmatopoietic organs is the intestinal wall, the source of supplies for the plasma, hence indirectly for the cells.

GASTRO-INTESTINAL DISTURBANCES are some of the most important causes of secondary anæmia, and perhaps of many cases in which the intestinal feature is overlooked.

In our cases of *severe diarrhœa* in men, in 60 per cent. the red count was not above 4,000,000; in women the counts ran higher. The real anæmia must have been more pronounced than this, for in some cases the blood was probably concentrated by the loss of fluid (one case with 7,900,000).

The leucocytes ran low (even to 2700 and 2500) in some cases, but above 10,000 in 30 per cent. of all.

Cabot mentions a case with 1,928,000 reds, another with 2,440,000 and 10 per cent. hæmoglobin.

In *chronic dysentery* the count is high or low. One case had

1,520,000 red cells, another 2,500,000. On the other hand, one (male) had 7,000,000 reds, 110 per cent. hæmoglobin, and 7000 leucocytes, and one (a woman) 6,300,000 reds.

In *chronic constipation* our cases showed normal or high counts, as would be expected.

Our cases of *dilated stomach* showed nothing abnormal as regards the leucocytes; for the most part the red count fell within normal limits, but four showed considerable anæmia (3,300,000, 2,400,000, 2,250,000 and 2,600,000). Those cases with the vomiting of large amounts of fluid should have a concentrated blood; all severe cases would be expected to show some anæmia of malnutrition.

Acute gastritis during the febrile period shows a slight leucocytosis, true of 70 per cent. of our cases of gastro-enteritis. A slight leucocytosis is also common in *chronic gastritis*, except the alcoholic form in which cases the counts may be quite low.

One case of *chronic dyspepsia* had a count of red cells, 1,960,000; hæmoglobin, 42 per cent. (index 1.1); leucocytes, 3600 (of which s. monos., 11.3 per cent.; l. monos, and tr., 0.3 per cent.; pmn. n., 85.6 per cent.; eos., 2.6; normoblasts, 2 per 100 leucocytes).

In *ulcerative colitis* counts below 3,000,000 are not rare.

In *amœbic dysentery* one would expect the count to be little affected since the intestinal lesion is so local, and severe anæmia is rare, yet in 24 per cent. there was a slight (4,000,000 to 4,500,000), and in 12 per cent. a more severe (2,200,000 to 4,000,000) anæmia. A leucocytosis was the rule (70 per cent. of cases) at some time during the disease, the highest count being 19,200. Futcher [66] found the general average of forty-three cases about 10,000. In children Amberg found an eosinophilia.

Anæmia of the Tropics.—It is said that Europeans after a stay of some time in the Tropics seem anæmic. Some consider this only apparent, and due to the distribution of the blood. The presence of basophile granulations in the red blood-cells, seen soon after the arrival there, and which were first described as related to malaria, would seem to indicate an injury to these cells. There are several tropical diseases, important causes of anæmia, which only now are we beginning to understand. These may explain some of the above cases.

Chronic Infectious Diseases.—Of these there are three which are most potent causes of anæmia,—lues, tuberculosis, and leprosy. While the toxine of the disease may be the most important element, yet the nutritional condition, especially the condition of the gastro-intestinal canal. the lack of exercise, and hemorrhages must also be included.

There is a great difference in toxines; in acute miliary tuberculosis without cyanosis, one of the worst septicæmias, there is little trace of blood destruction (see page 564).

[66] Jour. Am. Med. Assoc., August 22, 1903.

Anæmia is a common result of *pus formation,* and is due both to the toxines from the pyogenic organisms and to absorption from the pus focus of breaking-down tissue, and probably also to the over-taxation of the blood-building organs. The same is true in diseases with chronic *exudate formation. Albuminuria* is frequently cited as the cause of anæmia, and yet the actual daily loss of proteid to the blood-plasma even in a severe case is very slight, and could easily be replaced by one good meal. The poor condition of the digestive canal of nephritics is also important, but surely there is some toxine which has a deleterious effect upon the blood, as well as it surely does on the rest of the body functions. Dieballa has found a definite relation between the albuminuria and the hydræmia.

Spermatorrhœa, lactorrhœa, and diseases of the respiratory organs with a *large amount of sputum* are further causes. Yet cases with chronic purulent exudate formation maintain their blood condition surprisingly well, considering the drain there is on the blood, as in cases of chronic bronchitis and tuberculous abscess (see page 564). In all such cases it is the plasma which suffers first, the red blood-cells second.

In cases with marasmus there is an atrophy of the total blood which may cover an anæmia, while in other cases the anæmia may be more apparent than real, since there is a dilution of the plasma. At this point also may be mentioned the dilution of the blood from the absorption of effusions or other retained fluids. On the whole, the regulation of the blood is simply wonderful; for instance, after the removal of even seven litres of ascitic fluid at one time and its rapid reaccumulation the blood will show very little evidence of this enormous flux of fluid through the blood-vessels.

Fever is stated to be an important cause of anæmia, and yet it is not the elevated temperature but the toxines which cause the rise which also destroy the red cells, as evidenced by the increased hydrobilinuria. Most important are those cases of chronic cryptic septicæmia which for weeks may present the picture of severe anæmia without any suspicion as to the true nature of the trouble. On the other hand, **acute infections** will cause a rapid fall in the blood-count, as for instance Grawitz's case of streptococcus septicæmia, in which in a little over one day the reds fell from normal to 300,000.

A recent case of arthritis of unknown cause, but with blood-cultures negative, had a count which fell to, red cells 976,000; hæmoglobin, 17 per cent.; leucocytes, 4600. He improved rapidly.

In **yellow fever** considerable anæmia is found, in one case the count being 2,604,000, in another 1,400,500 (Maurel).

Pneumonia, diphtheria, scarlet fever, typhoid, acute articular rheumatism, smallpox, septicæmia, and other acute infectious diseases may cause a severe anæmia. The reader is referred to the various sections

on these diseases. In all cases, for the first few days at least, there may be no diminution in the red blood-count, even a hypercythæmia due to the concentration of the blood, seen best in diphtheria and typhoid fever, and which may cover a real anæmia. The rapid fall in the count which comes during convalescence or at the time of the crisis, as in pneumonia, is probably more apparent than real, and due to dilution of the blood resulting from the general vasomotor relaxation at that time (Grawitz); but the toxine of the infecting organisms may also be important by causing hæmolysis.

In many cases there is a drop in the count, but the quantitative changes are remarkably slight; only in very severe cases are microcytes, macrocytes or poikilocytes present. Hydræmia is the rule, the loss of albumin running parallel to the severity of the disease, and in severe cases reaching even 6.25 gms. of residue to 100 cc. of blood.

Intestinal Parasites.—Of these there are two famous as causes of anæmia.

Uncinaria Duodenale et Americana.—Historically this form of anæmia is most interesting, since the cases of miners' and tunnel diggers' anæmia due to this parasite were first rated as primary pernicious anæmia, at a time before the distinctive blood-features of the primary and secondary anæmias were understood; now it is claimed the picture can rarely simulate the pernicious type. This parasite occurs in many different countries and bids fair to prove to have been one of the most important causes of anæmia; it is now thought to be in this country the chief cause of the "anæmia of the South." Our one marked case during the past five years had a count of red cells of 2,424,000; hæmoglobin, 32 per cent.; leucocytes, 9700; eosinophiles, 5.6 per cent.; but in some epidemics the count falls below 1,000,000 cells.

The cause of the anæmia is disputed. That it resembles one due to hæmorrhage rather than to a toxine is seen from the small amount of iron in the liver, it being diminished even to one-quarter its normal amount, to the absence of a leucocytosis, and the very low color-index.

In our three cases of strongyloides intestinalis infection the blood showed: red cells 5,420,000, hæmogobin, 82 per cent., leucocytes, 6200; 3,560,00, 57 per cent., 21,500; and hæmoglobin, 60 per cent., leucocytes, 7500, respectively.

Bothriocephalus Latus.—This parasite is the cause of a most interesting anæmia. It is a tape-worm which may live for years in the intestine of a person whose blood is normal, and yet in other cases cause the most severe anæmia, the almost exact picture, both quantitatively and qualitatively, of the primary pernicious type, but which recovers after the worm has been expelled. In Lichtheim's case the red blood-corpuscles were 500,000; hæmoglobin, 20 per cent.; six

worms were expelled. In Schapiro's case the count was 837,000, and in twenty-three days after the worm was expelled, 2,975,000. Bezançon and Labbé give as the average of reds 1,300,000, and the limits from 395,000 to 2,150,000; those of the color-index 0.9 and 1.62. All the degenerations and other signs of a severe primary anæmia, poikilocytes, microcytes, macrocytes, the polychromatophilic degeneration, etc., are present. Even one-half the nucleated reds are megaloblasts, and yet in two weeks after the worm has been expelled the megaloblasts all disappear, and in three weeks the megalocytic blood returns to normal type, with even normoblasts gone. The leucocytes are normal both quantitatively and qualitatively. They vary from 3000 to 12,000 (Schaumann).

The reason for this anæmia is unknown, and yet when it does occur it is probably due to a toxine. It is not a loss of blood; it is not the presence of the worm alone, since but 16 per cent. of the hosts of this worm are anæmic. Askanazy says it is the time that the worm is in the intestine, but even this does not hold, and some cases are hosts twenty years before the anæmia begins. Schaumann emphasizes the predisposition of the patient. Dehio says it is the condition of the worms, only those worms which are diseased or dead causing the trouble; but the diseases of the worm are not always evident, and there are cases with a manifestly degenerated worm but no anæmia. Again, in other cases after the worm is expelled the anæmia is not cured; perhaps the ability for recovery has been lost. In this anæmia the iron of the liver has been found even twice normal in amount, which would indicate an intravascular destruction of the red blood-cells. The color-index is above normal.

Other intestinal parasites, Tænia saginata and soleum, Strongyloides intestinalis, in which counts as low as 760,000 have been reported (diarrhœa of Cochin China), Ascaris lumbricoides, are claimed, as occasional causes of anæmia, but the connection is as yet unsatisfactory.

Poisons.—Lead, mercury, arsenic, certain organic poisons, plant and animal toxines, ptomaines, and the toxines of burns, all may cause anæmia. Lead is an especially potent cause, both of the acute and chronic forms. While it is essentially a chlorotic anæmia, manifested first by the degenerations of the red blood-cells, their count being practically normal, it may be so severe that the count is reduced to even 1,300,000. Malassez says that there is a slight increase of diameter in the reds, their rigidity is increased, and that megaloblasts sometimes occur. The basophile granules are very common and important (see page 447). Whether the action of lead is directly upon the cells or upon the plasma first is uncertain, while some think this anæmia due to gastro-intestinal disturbances.

We have had during the past few years 17 cases. In 16 cases the lowest was 2,900,000; in 7 cases the red count was over 4,500,000; the mean, 4,200,000. Hæmoglobin, lowest, 38 per cent.; mean, 60 per cent. In 10 of 16 cases the leucocytes were above 10,000, maximum 25,000, but fell very soon after admission.

Long-continued use of certain of the coal-tar products causes a severe anæmia. Stengel and White [67] report a most interesting case, a woman with reds 2,092,000; hæmoglobin, 35 per cent.; leucocytes, 19,800 (a previous count), and 32,323 nucleated reds per cubic millimetre, of which 91.4 per cent were normoblasts, 3.5 per cent. megaloblasts and 5.3 per cent. free nuclei. The platelets were increased. There were many poikilocytes, a few basophile granules, and considerable polychromatophilic degeneration. It is interesting that the diagnosis of this poisoning was made from the appearance of the smear alone, despite the repeated assertions of the woman of the impossibility. It was found to follow the use of acetanilide. They mention Ehrlich and Lindenthal's case with nucleated reds in the proportion of 1 : 56 of the red cells. In Brown's case of acetanilide poisoning [68] at death the reds were 1,166,000, and the nucleated reds 22,150 per cu. m.m.

Splenic Anæmia is the name given to a group of cases with anæmia and idiopathic enlargement of the spleen. The anæmia is of the secondary type, the average of Osler's cases being over 3,000,000; there is no leucocytosis, or a reduced count. Such cases have profuse hemorrhage from the stomach and œsophageal varices. In one case in Osler's series the macrocytes and gigantoblasts were a marked feature of the case.[69]

Simple Primary Anæmia.—This form, which some separate from primary pernicious anæmia because of the differences in the clinical course, is also hard to separate from those secondary anæmias already mentioned as due to unhygienic conditions, poor food, hard work, worry, etc. It is a severe primary anæmia, characterized by the number of relapses, ending finally, however, in death. This type can be recognized only when the case is typical. It seems to stand midway between chlorosis and primary pernicious anæmia, some cases differing from the former only in the age of the patient, others presenting many features of the latter, and between them every gradation. Midway between these extremes is a group of cases with oligocythæmia and oligochromæmia of about equal grade, and leucocytes normal both quantitatively and qualitatively.

Progressive Pernicious Anæmia.—Eichorst's definition of this was a severe anæmia which in spite of all treatment progresses relentlessly to death. Pathologically, there is no lesion of etiology. The blood picture alone is not characteristic, for several varieties of secondary anæmia may assume a somewhat similar picture—" secondary pernicious anæmia." And yet the blood picture is so striking that the word " primary pernicious" now carries with it an idea of the blood picture

[67] Contrib. of the Wm. Pepper Laboratory of Clinical Medicine, 1903, No. 4.
[68] Amer. Jour. Med. Sci., 1901, vol. cxxi.
[69] Osler, Am. Jour. Med. Sci., January, 1900.

as well as its clinical and pathological significance. As illustrations of secondary pernicious anæmia are certain cases of cancer, phthisis, lues, malaria, repeated hemorrhage, lead poisoning, certain parasites, lesions of the bone-marrow, especially tumors, also osteomyelitis, atrophy of the gastric mucosa, stenosis of the pylorus, nephritis, certain rare cases of pregnancy, and purpura hæmorrhagica. In all the above cases there is a long history of anæmia-producing agencies, and this picture may represent the final stage, an almost complete bankruptcy of the blood-building functions, or the conditions may occur simultaneously.

The salient characteristics of the blood of primary pernicious anæmia are: Signs of rapid blood destruction (the degenerated reds, endoglobular degenerations, polychromatophilia, the urobilinuria, jaundice, the increased iron compounds (?) in the serum and the corpuscles, and the increase of iron stored in the liver and spleen); the poikilocytosis, a high color-index, and the megaloblastic blood formation. At first the poikilocytosis was supposed to be characteristic (Quincke); this idea was very soon corrected. Then the high color-index (Laache and Kahler), which some even now hold. A high-color index may occur in chronic cases of secondary anæmias with a very low blood count, but it is a very striking feature in all the primary cases. Ehrlich considered that megaloblasts were characteristic, but this also is not strictly true, although they occur especially here.

Volume of the Blood.—Clinically there is no way of determining the volume of the blood, yet from the appearance of the patient we are often sure it is diminished, and at the autopsy table are sometimes astonished at the small amount of blood in the heart and blood-vessels. We saw one remarkable case in Professor Müller's clinic, in which all the organs seemed almost exsanguine.

Gross Appearances.—The ear is a better place to obtain the drop of blood than is the finger. It may flow freely, or it may be difficult to get any. Lazarus considers that the former occurs when the patient is doing badly, and that the latter is evidence of improvement.

The blood is pale, of a light red watery color (Fleischwasser), and does not at all resemble blood.

We showed a tube full of this blood to a class on one occasion, asking them to tell from its appearance alone what fluid it was, and many of them said it was a cloudy urine, which, indeed, it did resemble.

The drop of blood is often streaked, evidence that the corpuscles have collected in masses. Cases have been described in which it is grossly of a coffee-color, probably due to hæmoglobinæmia. The coagulation time is often increased.

Red Blood-Cells.—In the fresh specimen these are seen to be few in number, and there is absence of rouleaux formation. The cells vary

much in size: many are slightly above normal, some very large; many are small, some very small. The cells do not many of them show Maragliano's endoglobular degeneration, but do that other degeneration, the accumulation of the hæmoglobin in the centre of the cell; most are of a uniform dark color. Nucleated reds will often be found in the fresh specimen. In a well-marked case the appearance of the fresh specimen alone will strongly suggest this disease. One has only to compare it with a specimen of normal blood, and the difference is striking.

COUNT.—An extreme oligocythæmia is the rule, and it is remarkable how few symptoms accompany these low counts, particularly as the volume of blood is also diminished. On the first visit the average cases will show a count of about 1,000,000 cells. Cabot's average was 1,200,000.

In our cases in the 102 admissions (several of the 81 cases being admitted more than once) the average first blood-count was 1,575,000. This is somewhat higher than that which other observers have reported, and is due to the fact that we had several cases admitted not for the symptoms of pernicious anæmia alone, but from attendant conditions, for instance, for nervous disorders. In 81 per cent. of our cases the count on admission was under 2,000,000 and in 12 per cent. under 1,000,000.

The count may go as low as 500,000, and yet the man remain comfortably at work, while others with four times the count suffer; evidence that the reason for the symptoms is not alone the oligocythæmia. Cabot thinks that the counts tend to stick at about 1,000,000 cells, dropping rapidly to this point and remaining there, then sometimes in improvement gaining rapidly to about 3,000,000; later to return to about this same figure. The count may remain stationary for some time, or it may diminish progressively until death. Quincke reported one case with a blood-count of 143,000, and yet recovery. Hayem's lowest case was 292,000, a fatal case. Scott's case had at death 268,000 reds; index, 2; leucocytes, 5900.[70]

After admission the count may continue to drop for a while, then to rise, or it rises at once, or it remains stationary.

It cannot be too often emphasized that a change in count may mean a change in the total number of red cells or a change in the volume of plasma.

An interesting fact already noted is that clinical symptoms seem to bear no relation to the red blood-cell count. Certain cases enter the hospital with a few symptoms and a blood-count of 1,500,000, while other cases are apparently in no worse condition and yet have a count below 1,000,000. The comparative comfort and physical strength of such patients is in marked contrast to cases of chlorosis and the secondary anæmias, which cases enter the hospital with the blood in an apparently much better condition. Again, in some of those cases in which the blood continues

[70] Am. Jour. Med. Sci., 1903, vol. cxxv. p. 397.

to fall after admission and then to rise, it is curious that the patient feels so well that he insists upon going home at a time when the count is no higher or very little higher than on his admission. In other cases in which the count rises after admission and then falls, death occurs when the count has reached the level of admission. In still other cases with an initial drop, as in five of our series, the count was rising at the time of death.

Although patients come to the hospital for symptoms which bear little relation to their blood-count, yet the same case on two or more admissions will enter with counts which are curiously close.

The red blood-count on the day of death in two of our cases was high—2,700,000 and 2,100,000; in three cases moderate, 1,031,000, 1,326,000, 1,216,000; and in thirteen cases, and this we think a hint of the blood picture at death due to this anæmia alone, the count was between 718,400 and 376,000, an average of 567,700.

The blood during intermissions is not quite normal. The red count is about 3,000,000, and the cells still large (Cabot). The color-index, however, is sometimes low, and the leucocytes increased by an increase in polymorphonuclears; nucleated cells disappear. The diagnosis now is important, especially to insurance examiners. In a recent case with almost normal blood the diagnosis was made by one examiner and the case refused. He succeeded in getting heavy insurance in another company, and died in about one year of this disease.

The VOLUME of the red blood-cells is best determined by sedimentation. The average volume, instead of the normal 45 to 50 per cent., is from 8 to 10 per cent., which seems high, considering the count, and is a measure of the large size of the cells. Capps found the volume index always above the color-index, hence the size of the cells explains satisfactorily the latter.

SIZE.—The average diameter of the red cells is somewhat increased, with, however, wide variations, the cells measuring from 4 to 13 microns, and with extremes beyond these. The average diameter may be 9 microns. In no other disease are there so many macrocytes. It is not the average but the mean size, or the percentage of macrocytes, which is of importance in diagnosis, since the microcytes will lower the average.

MACROCYTES.—Seventy per cent. of the cells may be very large, between 11 and 13 microns in diameter (Lazarus). In a case reported by Ewing 90 per cent. measured from 11 to 16 microns. Gigantocytes also occur. These large cells are less biconcave than normal, some are not biconcave at all. Some are oval; some seem flabby; they are often dark colored in the fresh, often polychromatophilic in the stained specimens; they never present the pessary form; some, however, are pale, according to Grawitz many are, but the dark color of large cells is, we consider, a quite constant feature, and in giving out fresh specimens of unknown bloods to a large class we find that they recognize this. Students should study fresh bloods from a large variety of cases, always making a specimen from normal blood for comparison, until

they will say unhesitatingly whether the size of the average cell is increased or not, and whether the color is darker or lighter than normal. The differences are more striking than one not accustomed to observe them would expect. In some cells there is a slight change of color shade as well as of depth. There are cases in which the blood is said not to be megalocytic. Macrocytes are not common in normal bone-marrow, and their presence here is considered (Laache) a compensatory attempt to replace the amount of hæmoglobin-containing protoplasm. Cohnheim first said it was reversion to the embryonic type. Ehrlich attributes it to a megaloblastic degeneration of the bone-marrow.

Microcytes.—These cells vary from 2 to 6 microns in diameter, and are usually of a deep color. They occur in large numbers also in secondary anæmias; they may fail here. So numerous are these cells in some cases that the average size of the red blood-cells is not above normal; hence the importance of judging the mean rather than the average size. The dark color of these microcytes may be due to their spherical shape, but they have sometimes a greenish tint, which would indicate a chemical change in the protoplasm. These microcytes might be suspected to possess amœboid motion, at least they change their shape and move quite actively among the other cells with an oscillatory motion. They have been described as monads, a leptothrix form, bacteria, and Hayem called them pseudo-parasites. It is very interesting to watch them in their movements. Such cells are pictured in Fig. 93.

Poikilocytes, formerly supposed to be characteristic, are relatively rare in secondary anæmia, but in this disease are often extreme both in number and in variety of shapes. Hook, raquette, spindle, and various dwarf forms occur. The sausage and the battledore shapes were formerly supposed to be found only here. The small forms show interesting contraction phenomena resembling amœboid motion (see above).

It is not unusual to find shadows among the deep-colored red cells. Since in a rather acute case abnormal cells did not appear until about two months from onset, McCrae suggests that such cells occur only after the condition has existed for some time.

Polychromatophilic Degeneration.—This is best studied in this form of anæmia. Although it is not always a sign of degeneration, in this disease for the most part it seems to be. With Ehrlich's triple stain these cells are a pale gray (Plate I, 25–28). With methylene blue they take a blue tint. Their number is almost parallel to the severity of the case (Grawitz). Red cells with Grawitz's basophile granules are very common, especially in severe cases, and have, Grawitz thinks, an important prognostic value.

Nucleated Reds. Normoblasts (Plate I, 29, 30, 35, and Fig. 113, a,

c, d, e).—These cells, described on page 478, occur quite constantly in pernicious anæmia, alone or with megaloblasts, and in especially large numbers during the blood crises. In a case of Bezançon and Labbé there were from 6000 to 10,000 normoblasts and 960 megaloblasts per cubic millimetre. Many of these cells show polychromatophilic degeneration, especially those in which the nucleus is dividing.

The *blood crisis,* so interesting a feature in cases of severe anæmia (see pages 479 and 534), is not always, as v. Noorden thought, a sign of very active regeneration and followed by a jump in the red count, although in secondary anæmia and chlorosis this may be the case. They may always indicate an attempt on the part of the bone-marrow to replenish the blood, but in some cases of pernicious anæmia they are followed by a fall in the red count, the convulsive attempt to stem the tide of destruction proving futile. Those followed by improvement occur especially in younger persons.

In some cases there are few or no nucleated reds in the peripheral blood. This means a slower regeneration. In other cases just before death all these cells disappear.

Megaloblasts (Plate I, 32, 33, 38, and Fig. 113, f).—These cells were first described by Ehrlich as characteristic of pernicious anæmia. They may, however, occur in any anæmia, and in any disease involving the bone-marrow. The difficulty in forming a judgment concerning their occurrence lies in the fact that cells which one man counts as megaloblasts others do not. Many criteria have been proposed for their recognition—the size of the cell, the size of the nucleus, the structure of the nucleus, etc. These cells being of such importance, we have made a rule in this clinic that whenever there is any doubt concerning a cell not to call it a megaloblast. One sees huge cells with a nucleus like a normoblast's; small cells with a nucleus like a megaloblast's; we reserve this name for cells which are large and have a nucleus correspondingly large. We do not consider any cell a megaloblast unless its nucleus is about the size of a normal red blood-cell; that is, about 7 microns. These cells are round or oval; they vary from about 11 to 20 microns in diameter. When very large they are called gigantoblasts. They are plump, often diffluent, and polychromatophilic. The nucleus in the fresh specimen has a well-defined chromatin net-work, but takes with the Ehrlich stain a pale greenish tint, staining so faintly that it may be overlooked; it is large, plump, round or oval, especially the latter; it is often surrounded by a clear circle, and outside of this circle the protoplasm often stains deepest; karyokinetic figures are sometimes seen, to find which some consider a grave sign. It is no easy matter to tell a polychromatophilic megaloblast with a pale staining nucleus from some mononuclear leucocytes, and, strange as it may seem, sometimes men of recognized authority differ whether to call a stained cell white

or red (Plate I, 36). Color, in a stained specimen, counts but little; the opacity of the protoplasm counts much, the red cell being more opaque. The point upon which most depends is the edge of the cell, for the spherical leucocytes must flatten out in the preparation, hence have a thin frayed margin, while red blood-cells, being disks, do not flatten, and have a thick, smooth, uniform, rounded edge, best seen when a cell touches another. Again, the edge of a leucocyte may overlap a neighboring cell, but the edge of the red cell merely flattens against it. There is no staining reaction which is characteristic for hæmoglobin, especially when basophilic, and the only occasion upon which one can be sure whether a cell contains any or not is when he sees it in the fresh state. If in doubt, it is more probably a leucocyte than a megaloblast, at least that is the safer view to take.

Although megaloblasts occur most commonly in this anæmia they are nevertheless rare here, and even Ehrlich, it is said, would hunt for hours until he found this much desired cell upon which he would stake the diagnosis of an otherwise clear case. If after a long search one finds six or eight such cells, he should be more than satisfied, and in some cases none will be found at times, and many on a later occasion. Their presence in large numbers is rare and ominous. Their number varies from day to day, there being interims of improvement during which they are absent, reappearing during a relapse. In other cases, however, they have been found during these periods of intermission while the blood-count is fairly normal. It is sometimes necessary to hunt two hours for one such cell, and yet the importance of this cell justifies that trouble. Upon the percentage of these rests some prognostic value. In megaloblasts polychromatophilia and the basophile granulation are particularly well marked.

Typical megaloblasts occur especially in pernicious anæmia, even in the mild grades in which cases they are of great diagnostic value. They occur rarely in other anæmias, except in the diseases of children. Their presence in bothriocephalus anæmia is well known; a few are found in cancer cases, especially those with metastases to bone-marrow. We have seen as beautiful a one as could be desired in simple tertian malaria without any marked anæmia, and they occur perhaps always in malaria of children. They occur in large numbers in splenomyelogenous leukæmia; in secondary anæmias and chlorosis very rarely. They would seem to occur especially in those conditions in which the bone-marrow is involved, and of this, apart from the anæmias, malaria is a good illustration. Some consider their presence an indication of the severity of the anæmia, not of the form; others that it is a sign that the bone-marrow is involved; others consider them to express an attempt on the part of the body to increase the volume of hæmoglobin-carrying protoplasm, and hence a protective measure; another origin

suggested is that they are swollen hydræmic cells, which are dropsical, containing the increased water of the plasma. It is true that some, the so-called "chlorotic cells" do suggest this very strongly, but they are always paler in tint; in cases of marked hydræmia there are often no such cells present, as, for instance, in nephritis, and they also occur in large numbers in chlorosis, and there the plasma is practically normal.

Intermediate Forms (Plate I, 31, 37, and Fig. 113, b).—This is a most troublesome term. In some cases this group includes nearly all of the nucleated reds found in the blood. We have made it an invariable rule that all cells suggesting megaloblasts and concerning which there is any doubt shall be put into this group. That is, a nucleated cell the size of a normoblast with the nucleus of a megaloblast, or a cell the size of a megaloblast with the nucleus of a normoblast, is assigned here, hence the group contains a great variety of sizes both of cells and nuclei. It is easy to see in studying the plates of cases published as pernicious anæmia that the many megaloblasts mentioned are cells which we have considered as intermediate forms; hence the confusion concerning their frequency.

Whether these cells are intermediate between megaloblasts and normoblasts we do not know; by the term we merely mean a cell concerning which we are in doubt. The existence of transitional cells has been denied by Ehrlich and Pappenheim. All transitions have been found by others (Schaumann). These occur in conditions in which megaloblasts would be expected. Some do not separate this group at all, and most consider them to have equal significance as megaloblasts. (See page 479.)

Microblasts.—These cells have a nucleus the size of a normoblast, but the protoplasm is exceedingly scanty and often ragged on the margin. The nucleus is usually pycnotic. Whether these cells are derived from normoblasts as degeneration forms, or whether they are preformed and have the same significance as normoblasts, is not yet known. They occur in pernicious, also in severe secondary, especially the post-hemorrhagic, anæmias.

The presence of nucleated reds was noted in 57 of 69 of our cases. In 13, definite blood crises were present; that is, more than 50 nucleated reds per 1000 leucocytes. This is rather an arbitrary line, and yet we have found that, in our cases at least, it corresponded quite well with the blood pictures. In all cases normoblasts occurred, while in 40 (58 per cent.), megaloblasts also. In the other six cases normoblasts and intermediate forms occurred.

In these 57 cases there were 63 periods during which nucleated reds were present. Of these 63 periods, in 26—that is, in 41 per cent.—there followed a gain in the red blood-cells; in the rest, either no gain or a loss. Of 14 periods without nucleated reds, during 8 there was a distinct gain.

In 13 of our cases (19 per cent.) blood crises were present. Five of these cases died. Of the 50 or more nucleated cells per 1000 leucocytes constituting the crises, the normoblasts varied from 5 to 3128; the intermediates reached even 212, and the megaloblasts 44. There seem to be two definite forms of blood crises, those

in which normoblasts largely predominate and those in which the intermediate and megaloblasts are also present in considerable numbers.

It is the normoblastic crises particularly which are followed by a rise in the red count; those with many megaloblasts are less inefficient or occur in a condition of the bone-marrow when it cannot regenerate the blood. They appear especially when the patient is losing ground.

The most remarkable blood crisis lasted for nineteen weeks. During this time the red blood-cells, at the beginning 1,902,000, rose to 2,562,000, and then finally dropped at death to 1,328,000. During this time the leucocytes varied from 3000 to 5000 until the day of death, when there were 16,000. During the whole period the number of normoblasts per thousand of leucocytes was almost always above 500, reaching on one occasion 1164, a little later 1032, and finally 3128. On this day the leucocytes were 4600; hence the total number of normoblasts per cubic millimetre was 14,388, of intermediate forms 460, and megaloblasts 138 per cubic millimetre.

Hæmoglobin.—The hæmoglobin is much reduced, rarely above 50 per cent., and often as low as 10 per cent. The color-index is normal or high, a point of the greatest importance in determining the nature of the anæmia. In our cases on admission the hæmoglobin averaged 34 per cent., and the color-index in 80 per cent. of the cases was over 1, an average of 1.1, and in 2 cases as high as 1.9.

Ewing considers that the index is low in the chronic cases and high in the acute. For it to rise is considered a bad sign, indicating as it does a falling count; with improvement there is always a lowering of the index due to the newly formed cells, which are of lighter weight than normal.

This high color-index has been ascribed to a "globular richness," *i.e.*, the corpuscles contain more hæmoglobin per cell than is normal, a protective provision, in favor of which the corpuscles have been estimated to be of almost twice normal weight; to the number of large cells, and the hæmoglobin does run parallel to the number of megalocytes; again, and with good reason, it has been considered, in part at least, only apparent, the explanation being that many microcytes are overlooked in the blood-count, yet they altogether contain no small amount of protoplasm; and, lastly, the presence of hæmoglobinæmia. That the chemical nature of the red blood-cells is not normal, many insist; the nitrogen of the cells has been found increased (v. Jaksch), hence the name "hyperalbuminæmia rubra;" it has been estimated that more iron is sometimes present than albumin to complete the hæmoglobin molecule, hence either the iron is increased in the hæmoglobin molecule, or is present in other combinations. Additional evidence for this is the hæmatogenous jaundice, and the iron compounds in the plasma, although the presence of these latter bodies is considered by some as simply evidence of poor technic. Taylor considers the high color-index as an optical illusion unsupported by chemical analysis; Capps, that color-index never surpasses volume index; Grawitz (and this point appeals to us very strongly) warns against hæmoglobin determinations with an ordinary hæmoglobinometer and emphasizes the error of overlooking microcytes in blood-counting. He considers that the best test of the index is the visual, and finds by this that many cells are of normal size and have good color, while others are paler than normal, hence considers the inequality in the distribution of protoplasm and the production of poor cells the prominent features of pernicious anæmia. Bezançon and Labbé think that from their appearance one does not get the idea that the cells are overrich in hæmoglobin. It is our opinion that they are. Many of the large cells in fresh specimens will be seen, when compared with normal ones, to be of a darker tint, but whether this tint is

due to an increased amount of hæmoglobin, to the greater optical thickness of a spherical cell, or to chemical changes of the hæmoglobin, it is difficult to say, and perhaps all elements enter. These large cells show little biconcavity, and often appear somewhat spherical. That changes in the hæmoglobin can make the red blood-cells appear darker is seen in many degenerating cells, in cells picked up by phagocytes, and in malaria, which cells have a somewhat darker and slightly greenish tint.

What is most important, the ordinary hæmoglobinometers which use a color prism do not give accurate readings in the lower half of the scale, and an error of 5 per cent., so insignificant in normal blood, changes the index considerably when added to a total of 10 per cent.

The hæmoglobin during the course of our cases ran parallel with the red blood-cells, as a rule. Yet due to the tendency to form large cells, as the case becomes worse the index slowly rises, and at death averaged 1.5.

Leucocytes.—In severe and uncomplicated cases there is always a leucopenia. Cabot's average was 3800, and in 72 of 110 cases below 5000. The leucocytes in our cases on admission averaged 4600. This includes all cases, even those with a leucocytosis due to complications. In 75 per cent. of the cases the count was under 5000. They may go as low as 1500 or 2000, and sometimes before death as low as 500 per cubic millimetre. Their number runs parallel to that of the red blood-cells, as a rule. A leucocytosis means either a complication, as pneumonia, some pus process, a blood crisis, in which case the large number of leucocytes may even suggest a leukæmia; and lastly, at death, the picture may be leukæmic (100,000).

Very roughly the leucocyte count ran in our cases parallel to the red count, and at death varied from 660 to 16,000, and averaged 5950.

Of our 81 cases, in 55 (70 per cent.) at some time during their stay the count fell below 3000; in 32 (40 per cent.), below 2000; in 9 (11 per cent.) it fell to 1000 or below. The very low counts, 1000 or below, are found only in the severest cases.

The percentage of *polymorphonuclear neutrophile cells* is roughly parallel to the total leucocyte count. This is best seen in the rise of these cells with the improvement of the case. Their percentage is lowest in the low counts, and the low count seems to be due to their diminution.

The percentage of the non-granular mononuclear cells varies inversely to that of the granular cells. The highest in our cases was 93 per cent. In the majority of the cases the percentage relation of the leucocytes tends to be constant whatever the total count of these cells, and indicates that these variations are due more to the distribution or dilution of the blood, perhaps stasis in the vessels, than to any real change in blood formula. On the other hand, there are considerable changes in total count, in which the absolute number of these mononuclear non-granular cells is quite constant.

Toward death the percentage of these cells rises, probably because the granular cells are formed in diminishing numbers. It is interesting

that the variations in the percentage of these cells show definite waves during the course of the disease.

The relatively high *lymphocyte* count is seldom a true lymphocytosis, but is due to an absolute decrease in the polymorphonuclear cells. The average of small mononuclears is 45 per cent. It may reach as high as 62 per cent. and before death even 79 per cent., and yet the absolute number be normal. This has been considered evidence that these lymphocytes arise in the lymph-glands and not in the bone-marrow. This decrease in the polymorphonuclear cells is the important feature, and in diagnosis excludes often cancer and septic anæmia, yet in the same case the count varies so much that it is not of very much importance.

In 12 (17 per cent.) isolated counts there was a true lymphocytosis. This was maintained in no case for more than one or two counts. Two of these were cases with a definite leucocytosis, while in the other cases the total count was not above normal limits. Hence a lymphocytosis may occur, but is not a common feature.

The *eosinophiles* averaged about 2.7 per cent. They may reach as high as 9 per cent., and are often absolutely increased. In extreme cases they may be diminished.

The *myelocytes* may reach 2 per cent. Their presence is more constant and their number greater than in any disease except leukæmia. In acute exacerbations of the disease they may reach even 29.4 per cent. of a total of 34,000 cells (Billings). Eosinophilic myelocytes sometimes occur, but rarely. In 23 of our cases myelocytes were present in numbers varying from 0.2 to 8 per cent. Nine of these cases were fatal. In 12 cases the percentage was not above 1 per cent. In 6 it was above 3 per cent.

In our cases the myelocytes occurred especially under two conditions; in cases with a very low count, even the lowest, in which case their percentage was the highest; for instance, of 1800 leucocytes, 8 per cent. were myelocytes. Again, they were much increased in cases with leucocytosis, as, for instance, a case with 14,400 leucocytes and 2 per cent. myelocytes; another with 11,600 and 0.5 per cent. myelocytes.

Mastzellen were present in 29 of our 69 cases. In 2 they were over 3 per cent., but in 8 over 1 per cent. Of the other 21 cases the average was 0.5 per cent. If these high percentages were really of Mastzellen it would show that in pernicious anæmia they show a definite increase hitherto not mentioned. We doubt very much that this is the case. In our cases of pernicious anæmia, in none were differential stains for these cells used, and in this disease it is quite common to find polymorphonuclear cells presumably of the neutrophile series without granules. So marked is this in some cases that it has been suspected that the body has lost its ability to form the neutrophile material (Ehrlich). This may

explain their high percentage. As is well known, in using Ehrlich's stain most non-granular polymorphonuclear cells are counted as Mastzellen. The high percentages occur always in cases with a low total count, the average of the above cases with over 1 per cent. being 3900.

Degenerated leucocytes are common; pale, swollen, and vacuolated, with the nuclei fibrillar. There is an increased number of neutrophile granules in the periphery of some cells. Hayem considers that they will imbibe a certain amount of hæmoglobin. Certain cases are reported in which the diagnosis between pernicious anæmia and acute leukæmia was said to be quite difficult; one, for instance (Williamson and Martin), in which the red blood-cells were 300,000, hæmoglobin 12 per cent., leucocytes 38,000, with the small mononuclears 99 per cent.; Westphal's case, with 816,000 reds and 24,000 leucocytes; Bezançon and Labbé's, with 550,000 reds, of which 3520 per cubic millimetre were nucleated, leucocytes 32,000, small mononuclears 66 per cent. (see page 552).

The absolute number of the eosinophiles is a splendid index of the course that the blood is taking, running in many cases parallel to that of the red blood-cells. During sixteen admissions there was a definite rise of these cells attending the improvement of the condition.

In several cases, with little change in the condition of the blood, the number of these cells was fairly constant, while in 10 cases these cells fell as the red blood-cells dropped, in 3 being absent at the time of death, and almost so in 2 others. These cells may drop as the patient goes down hill, even though the red blood-cells do not. Some cases are exceptions, as, for instance, in 8 there was no rise in eosinophiles as the blood improved; in 4 there was a rise, but without any accompanying improvement; while in one count, a terminal pneumonia, these cells were 220 in number at death. The explanation of these exceptions, however, is not difficult. In cases in which with apparent improvement there was no rise of eosinophiles it is to be noted that the increase in red cells was particularly rapid, averaging 43,000 per day. In 5 cases, with a considerable rise ending in a definite eosinophilia, the average gain of red cells per day (17,000) was slow, and lasted over a considerable period of time; while in those cases with a slight increase and not ending in a definite eosinophilia the rise in the red count was still more rapid, averaging 34,000 cells per day. This, we think, indicates that the slow rise of the red count over a considerable period of time is more surely due to new blood formation. The rapid changes in the red count may mean plasma changes, etc.

The number of eosinophile cells may not run parallel to that of the red cells, but their large numbers occur chiefly in those cases which are doing well or after they have already done well; that is, following

a rise of the reds these cells may be increased. They are present in particularly large numbers in those cases gaining very slowly.

A diminution in their absolute number may be of ill omen, as in one case with six counts during a period of fifteen days ending in death the red blood-cells remained constant; that is, the first count, 2,832,-000, the last 2,704,000, and the average in all 2,700,000. The absolute number of eosinophiles at first was 183, shortly afterwards 180, or about the same, and toward the end none at all.

Platelets.—The blood-platelets are decreased or even absent, often only one-twentieth the normal number. In other cases they are said to be increased (v. Limbeck and Sahli). Grawitz considers that they vary. Hayem found the count as low as 25,000, or even 15,000, per cubic millimetre.

Coagulability is usually decreased. The blood from a venesection does not separate into clot and serum.

Serum.—The changes in the plasma are important, since this constitutes 90 per cent. of the total blood. It loses very little of its albumin; for instance, there is a loss of 50 per cent. of the albumin of the total blood yet of the serum of only 8 per cent. This is very different, therefore, from the hydræmic anæmias after hemorrhage and those due to poor diet, in which the serum is most affected. It is an important diagnostic point also to exclude the anæmias of cancer and of sepsis (cryptogenetic infections).

The **specific gravity** averages about 1030, and may go as low as 1025.

The **solids** are very low, averaging about 9 per cent. The water is increased even to 90 per cent. The greatest loss is in the albuminous bodies, they being reduced even to one-third normal. This is especially due to the corpuscles, for the serum in the severe cases may be normal. In the plasma the serum globulin is alone decreased, serum albumin being practically normal.

Chlorosis.—This is a disease especially of young girls at puberty, the essential blood-feature of which is a reduction in the hæmoglobin. The count of red cells is almost normal; the cells show few signs of degeneration or destruction; the hæmoglobin formation is very defective, and there seems to be a polyplasmia. It is the only good illustration of anæmia due to defective hæmogenesis, and differs from all other forms in the lack of evidence of blood destruction (v. Noorden), as shown by the poverty of the urine in pigment, the slight degeneration of the red blood-cells, and the absence of jaundice. Chlorosis is more a clinical than a blood picture, since this latter is well simulated by many secondary anæmias, for instance an ordinary post-hemorrhagic case, and we cannot make a diagnosis from the blood alone, since all secondary anæmias have some of its features. Yet clinically the picture is sharp,

so sharp that some will diagnose chlorosis without blood changes (Laache).

The blood features to be emphasized are: that in chlorosis there is a more uniform diminution in the size of the red cells, and a more uniform paleness, while in the secondary anæmias, even of a severe grade, the red cells vary widely in size, and a good many will be normal in size and color; in chlorosis the color-index is lower, a lymphocytosis more common, the nucleated reds more infrequent, and coagulation more rapid than in secondary anæmias.

The gross appearance of the drop is very pale, thin, watery, and the blood clots rapidly.

The count of the RED BLOOD-CELLS need not be very much reduced, and yet in over 60 per cent. of the cases it is under 4,000,000 cells at the first visit (Reinert, v. Limbeck). Thayer's average of 63 cases at the first visit is 4,096,000; Cabot's, 4,112,000; Gräber's, 4,482,000, while that of Grawitz is from 3,400,000 to 4,300,000. The minimum count of Cabot's was 1,932,000; of Thayer's, 1,953,000, and of Hayem's, 937,360. Gräber, who claimed that in simple chlorosis there is no diminution in the count, and that this would mean a complication, cites a maximum of 5,700,000 cells. Low counts are rare, and some complications may always be suspected, as ulcer of the stomach. The color of the red blood-cells indicates a marked diminution of hæmoglobin, their biconcavity is pronounced, the pessary form of cells common, and they stain very poorly (Plate I, 23, 24).

There is a quite uniform diminution in the size of the cells, and yet the large, pale, so-called "chlorotic cells" bring the average up to almost normal, the cells varying from 5.2 to 11.5 microns in diameter, with an average of 7.5. These large chlorotic cells are interesting, since many consider that they are dropsical cells,—that is, cells swollen because of the water they have imbibed from the plasma; and yet their number is usually few and the great majority of cells are almost uniform in size, and a little smaller than normal. There is not the large admixture of normal cells seen in secondary anæmia. Macrocytes are rare. Microcytes are more common. Schaumann and Willebrand say that at the height of the disease the small cells predominate, while during convalescence the large cells. Grawitz says the cells are largest when the case is at its worst. These large chlorotic cells may be very numerous, even one-third of all, at the height of the disease.

Poikilocytes and degenerated cells are rare except in the severer cases, and the polychromatophilia is considered by many to mean youth of cells, and, therefore, to be a sign of active regeneration (Grawitz). "The granular degeneration does not belong to the picture of chlorosis, but means some complication." Stengel and Pepper think it common.

Nucleated reds are very rare except in the severer cases, or during improvement, when blood crises may occur (some deny this). They are much rarer than in the secondary anæmias. They are usually of the normoblastic type, rarely megaloblast.

HÆMOGLOBIN.—It is the reduction of the hæmoglobin which is the characteristic feature. This may be reduced to even 20 per cent. Cabot's average on first visit was 41.2 per cent.; Thayer's, 42.3 per cent. The color-index is, therefore, low, averaging 0.5, but in some cases it is as low as 0.3. Secondary anæmias never reach this level. The cause is the small amount of hæmoglobin in each cell and the large numbers of small cells. The volume of the red blood-cells is just about half the normal.

The average *leucocyte count* in Thayer's cases was 8467; in Cabot's, 7485; that is, the count is normal; a leucopenia is not uncommon. This is important in diagnosis, since in the secondary anæmias, especially those of cancer, there is usually slight leucocytosis. The leucocytes may increase faster than the red cells during the convalescence, hence then give rise to a leucocytosis.

Grawitz and v. Limbeck say that the blood formula is normal. Most observers, however, even in mild cases, find the small mononuclears about 33 per cent. There is a slight absolute diminution in the neutrophile cells, and among them may be found cells approaching myelocytes, but typical myelocytes are very rare. The eosinophile cells are usually somewhat increased, averaging 3.5 per cent., and in some cases even 9.6 per cent.

During the last five years there have been admitted to our female wards but 13 cases diagnosed as chlorosis. Of these, but 2 were at puberty, and the rest from seventeen to twenty-five years old (relapses?). Of these, the lowest was 2,600,000, the highest 4,000,000, mean 3,700,000. Hæmoglobin, 26 to 49 per cent. Color-index, 0.36 to 0.63; mean, 0.47.

Leucocytes: lowest, 2400 and 3800; between 5000 and 7000, 6 cases; highest, 8000.

Differential counts made in 7 cases, all practically normal (even in the case with a total of 2400, there were small mononuclears, 17.2 per cent.; large mononuclears and transitionals, 3.9 per cent.; polymorphonuclears, 77.1 per cent.; eosinophiles, 1.8 per cent.).

It is interesting that when they left the hospital all (9 cases thus examined) had gained practically the same, only between 900,000 and 1,710,000 cells; mean, 1,100,000.

The explanation of the decrease of typical cases of chlorosis is a much mooted one, but we suspect it is the vast increase of patent medicines during the past ten years which contain iron.

The PLATELETS are increased, as a rule; in fact, in no condition are they as numerous as here. They also are large in size.

The *specific gravity* of the blood is low, sometimes reaching 1030. This is due to the loss of hæmoglobin, and in this disease alone does the

specific gravity run parallel to the hæmoglobin content. Grawitz states that it varies from 1035 to 1045; others put the figures at 1030 to 1050. Grawitz says that if it is under 1035 there is some complication.

The *alkalinity* of the blood is normal.

The *isotonicity* of the cells is low.

In the *serum* there is very little change, since there is no blood destruction, a feature which usually affects the plasma first. There is no hydræmia in chlorosis, yet the total plasma seems increased (polyplasmia). As the case improves, the number of the reds rises rapidly to normal; that is, "the anæmia is first cured" (Gräber), then more slowly are these light-weight cells replaced by those more normal in size and shape and in hæmoglobin content. Yet these variations in the count need not mean a new formation of cells alone, since the plasma changes must not be neglected; in fact, the first sign of improvement is an increase of specific gravity and an increased count due to the disappearance of some of the plasma, as shown by the polyuria and the disappearance of œdema. Later the signs of regeneration appear, also the gradual elimination of the faulty cells, the appearance of more normal ones, and the rise of leucocytes to even above normal.

Leukæmia.—Leukæmia is a disease marked by the constant presence in the blood of granular mononuclears or an increase of the non-granular cells with round nuclei, the immature cells of the blood-building organs which are not normally present in the peripheral blood. The blood formula is markedly changed. There is, as a rule, also a great increase in the total number of the leucocytes, and yet during the periods in which the count is normal the diagnosis can often be made from the large numbers of these abnormal cells present.

Leukæmia is rated among the primary anæmias, although the diminution in the red count is not an essential feature. The reason perhaps for the emphasis upon the anæmia is the old view that these white cells were immature red cells which had failed to develop hæmoglobin (Virchow). Interestingly enough, for certain cases of acute leukæmia there is now a reversion to this idea by some writers, the similarity in shape of the normoblasts and the lymphocytes, and the percentage relations both in the marrow and in the blood indicating that perhaps the mother cell of both is increased and produces the white cells chiefly.[71] Apart from this, the cachexia, which always arises sooner or later, is a very important feature of the disease.

Anatomically, it is a disease with lesions of the hæmatopoietic organs only. Formerly it was the pathological picture which was the most important, then the blood picture; as a fact, both are important, since the latter is not the disease, but a symptom of the former.

According to the blood picture three forms may be separated:

[71] See Reed, Amer. Jour. Med. Sci., October, 1902.

(1) Lymphatic leukæmia, "lymphæmia," in which the increase is of the non-granular cells.

(2) Splenomyelogenous leukæmia, "myelæmia," or "true leukæmia," with an absolute increase of all forms, but especially with the presence of the mononuclear granular cells.

(3) Mixed leukæmia, in which both the granular and non-granular mononuclear cells are increased.

Of all three forms may occur cases of acute leukæmia (see page 552).

Whether the disease is a disease of the lymph-glands on the one hand, and the bone-marrow on the other, or whether both non-granular and granular cells arise in the marrow alone, is a question for the physiologists to decide. From a hæmatological point of view it is to be emphasized that the abnormal cells are always young forms; that for many of the non-granular, mononuclear cells the name lymphocyte is a misnomer; that in the myelogenous and mixed leukæmias all forms of leucocytes are involved; and it is only in the so-called lymphatic leukæmia that one group alone is increased.

Leukæmia differs from leucocytosis not so much because of the higher white count as of the presence of large numbers of these unripe cells, and also of its chronicity. In the intermissions the diagnosis can often still be made from the differential count.

There is a tendency now to group all forms in one, and pathologically this may be justified, but not clinically, for there the line is generally quite sharp between the myelogenous and lymphatic forms, although some (Wolf especially)[72] claim to have demonstrated the transition from a lymphatic to a myelogenous type, and the reverse change seems to be even more common. In claiming such transitions, Wolff insists that cases in children must be excluded; a coexistent leucocytosis and lymphatic leukæmia must be thought of; and considers that one may recognize non-granular myelocytes. Grawitz sums up the question thus: that leukæmia is one disease, with various symptoms and various blood pictures, and with but one law, and that is its lawlessness.

Splenomyelogenous Leukæmia (Plate I).—In this disease there is a marked increase of all the granular cells, especially neutrophiles, also of eosinophiles and basophiles, and especially of the young forms of these cells with spherical or but slightly indented nuclei. The non-granular cells are also very much increased.

Total Blood.—In many cases of this form of leukæmia there is certainly an increase in the total volume of blood, as is shown before death by the dilatation of the veins, as well as at the autopsy table. Later on, however, in other cases, as death approaches, a diminution in the total blood volume seems to occur.

[72] Zeit. f. klin. Med., 1892, vol. xlv.

Grossly, the blood looks normal even when the leucocytes are almost equal in number to the red blood-cells. In extreme cases it has a pale, more opaque look, and flows sluggishly. Cases have been described in which the fresh drop resembled "chocolate mixed with cream." This must be very rare, as so many have never seen it. It is probably due to hæmoglobinæmia. When making smears the blood seems thick; hence it is hard to get good preparations (which appear granular); the diagnosis has often been made in this way. If the blood be allowed to settle and coagulate, there will form a grayish-white layer on the top of the clot, which may suggest the diagnosis. Coagulation is slow, and in the severe cases sometimes absent.

Red Blood-Cells.—As a rule these are diminished (Grawitz said always unless some factor concentrating the blood was present). In Taylor's cases there were none above 4,000,000. Very rarely is the count normal; very rarely is it much diminished, and yet sometimes it is. The cachexia, slight jaundice, increased urinary pigment, and the deposit of iron in the various organs show that a toxic hæmolysin is present. Cabot's average count was 3,120,000; Osler's, 2,850,000. In 9 recent cases with 11 admissions, the lowest count was 1,640,000; the highest, 3,800,000; mean, 2,800,000. As the leucocytes increase the reds decrease, and *vice versa*. There are exceptions, however, and the count may remain almost normal for a long time, some cases at 5,000,000. The anæmia may be due partly to the hemorrhages, which are so common, or to the albuminuria and the diarrhœa. The count may be almost as low as in pernicious anæmia, and attention is called to the fact that this oligocythæmia persists during those periods at which the leucocyte count is normal and the patient feels better. If the patient is seen for the first time at this period, the diagnosis of pernicious anæmia would certainly with justice be made (Taylor). The subjective condition of the patients depends little on the count of red cells, since they are ready to go home when this has changed but little.

QUALITATIVE CHANGES.—The anæmia is of the chlorotic variety, the cells being pale, with little hæmoglobin. This is best seen if stained with indulin or nigrosin. There is remarkably little degeneration, although the endoglobular areas do occur. Microcytes and macrocytes are rare; poikilocytes occur in all cases, but not many as a rule. The polychromatophilic degeneration and the basophilic granules are common, yet in some severe cases the reds are perfectly normal, and when at the most they are still not numerous. Biermer's test was found positive in two cases.

NUCLEATED REDS.—These cells are remarkably numerous considering the mild grade of the anæmia; in fact, this is the condition *par excellence* in which to study them, as there is no disease in which normoblasts occur more constantly; yet their absence is not against

this diagnosis. Megaloblasts are also common and sometimes many in number; also gigantoblasts, which may reach 20 microns in diameter; yet this megaloblastic feature is not so marked as it is in pernicious anæmia. Microblasts occur. Karyokinetic figures are common in all stages of division, and are best studied here. Of Taylor's 16 cases, in 2 the number of nucleated reds varied from 60,000 to 70,000 per cubic millimetre, and one of the first effects of the arsenic was to reduce the number of these cells. At the end there may be but a few or an increased number of these cells. It is of interest that accompanying marked rises in the white count the nucleated reds rise roughly parallel.

The **hæmoglobin** is reduced, the color-index being about 0.6. Osler's average of hæmoglobin was 42 per cent. In 9 recent cases the mean was 30; index, 0.54. This is hard to determine, since the leucocytes render the blood so opaque that many of the figures may have been wrong.

Leucocytes.—From the appearance of the fresh specimen the diagnosis may sometimes be made at a glance, not from the large number of leucocytes, but from the large number of immature cells normally not present in the blood. One may have a simple leucocytosis as high as some cases of leukæmia, cases of leukæmia with normal counts, and some post-febrile cases with a blood formula which for a time suggests leukæmia.

Counts of 500,000 are not rare. Cabot's average at the time of the first visit was 438,000; Osler's, 298,700. The counts vary, yet not as much during one admission as would be supposed, the daily counts maintaining approximately the same level for weeks, and during the same day we have not seen the great variations spoken of (*e.g.*, on one day the blood was counted each four hours: 146,000, 134,000, 141,900, 143,200). Some are cases with quite uniformly high counts—over 400,000; but the most have moderate counts,—from 100,000 to 300,-000 (63 per cent. of 51 cases), while fewer are below 100,000. One case may belong at different times to each of these groups, but during one hospital admission it keeps at a fairly constant count. There are periods when it is even normal, yet with the formula pathological as a rule, although not always. (In three of Taylor's cases there were no qualitative changes.) In some other conditions neutrophile myelocytes occur, but not with increased eosinophiles and basophiles; while mononuclear eosinophiles are rare in other conditions.

Differential Count.—All the cells of normal marrow appear in the blood. Among these are *neutrophile myelocytes* in enormous numbers, usually the predominating cell. The very large myelocytes occur, with a large chromatin-poor nucleus which stains palely, is hard to make out, and is often in an eccentric position; these cells are seen only

here and in some diseases of children; they are sometimes even 30 microns in diameter (Cornil's myelocytes). Also smaller myelocytes, about the size of an ordinary leucocyte are seen, with a centrally placed round nucleus which stains well. Lastly, dwarf myelocytes about the size of red cells. All transitions between these largest and smallest myelocytes may be found. Mitoses are more or less common. The number of granules in these cells varies considerably, some being full, in others there are a few, and still other cells are confusing, since different persons will not agree as to whether they are granular or not. Grawitz emphasizes the large non-granular cells, some of which are very large, with a homogeneous body and a large pale nucleus which is often seen without protoplasm, and which break up rapidly; others are medium sized, with basophilic protoplasm staining intensely, and a medium-sized nucleus; in other similar cells beginning granulation can be seen. These are the transitional forms of myelocytes. There are also myelocytes about the size of a leucocyte, with rather small compact nuclei. This is the form seen in leucocytoses, etc.

Eosinophile myelocytes are found, sometimes in good numbers, but never as many as of the above. There also occur all transitional forms between these and eosinophile leucocytes.

Polymorphonuclear Neutrophiles.—While these are relatively diminished (Cabot's average 46 per cent.), their absolute increase is considerable, even to about 50,000. Anomalous cells are common, some very large, even 20 microns in diameter, some small, or dwarf cells, 4 microns in diameter, a variation in size which never exists in leucocytosis. Again, cells with unusually shaped nuclei occur, and cells with more than one form of granule. The granules may vary in tint, which depends partly on the method of fixing; in one case all cells were described as non-granular. Free granules can be found in the plasma from the many cells which have broken down.

Lymphocytes.—The percentage is reduced, averaging 10.6 per cent.; there is usually an absolute increase, yet sometimes not. These cells vary much in size, and among them are some which it is very difficult to tell from myelocytes. Others are the large mononuclear cells, common enough in the marrow, but which never reach the blood normally or in other diseases. Some have very irregular shapes, some a few granules.

The *large lymphocytes* have a scanty ragged protoplasm and a large chromatin-poor nucleus. These are Fränkel's unripe cells, supposed to be characteristic of acute leukæmia, but occurring also in the chronic types. *Large mononuclears,* both those of the normal blood and those mistaken for myelocytes, occur in large numbers. Of the latter the nucleus is often very basophile, the protoplasm is finely fibrillar, and

distinctly basophilic or acidophilic. These cells before the Ehrlich's stain was used were reckoned as myelocytes.

Large phagocytes (splenic cells?) are sometimes present, numbering in one case 1.2 per cent. of the leucocytes (total count 216,000).

Eosinophiles.—In this disease there is usually an absolute increase of these cells. Ehrlich, indeed, stated that he would not make the diagnosis of leukæmia unless an absolute count of more than 250 cells was present. Since then three cases at least have been reported[73] of undoubted leukæmia, but at times without a single eosinophile cell, and others with extreme fluctuations in their numbers. As a rule the minimal number in leukæmia is about 3000, the average percentage 5.1, and the average absolute number 11,000. These cells occur in all modifications like their neutrophile analogues, the large myelocytes (formerly said to be the characteristic cell), the medium-sized ones, the dwarfs, and the ordinary leucocytes. The eosinophile myelocytes may in this disease form the majority of the eosinophile cells.

Basophiles.—Ehrlich considered that there was always an absolute increase of the Mastzellen in leukæmia, and that this was the only condition in which they were increased. Their absolute increase may be above that of the eosinophiles, and is always proportionally higher. In one of Lazarus's cases they reached 47 per cent.; in one of Cabot's, 10 per cent., while Taylor mentions a case with an absolute count of basophiles of 140,000. Taylor also states that in two cases no Mastzellen were present.

Charcot-Leyden crystals may be found in the blood after it has stood for a while, but also in the fresh blood, as was shown by splenic puncture. Some observers, however, including v. Limbeck and v. Jaksch, have never found them. They are normal in the bone-marrow and are present wherever the eosinophile cells are increased. Bezançon and Labbé say that leucin spherules also will separate spontaneously.

Ehrlich considered that from the examination of the smear alone the diagnosis of this form of leukæmia could be made. The six points which he emphasized were: the presence of neutrophile myelocytes; eosinophile myelocytes; an absolute increase of eosinophiles and of Mastzellen; the presence of atypical cells, among which are dwarf eosinophiles and neutrophiles, both mononuclear and polynuclear; cells in mitosis; and, lastly, the large number of nucleated reds. As mentioned above, however, the absolute number of eosinophile cells is no necessary part of the blood picture. And, indeed, any one of these points may fail for a while, at least.

A great many of the leucocytes show signs of extreme degeneration. Ewing considers that eosinophile myelocytes with granules of

[73] See Simon, Am. Jour. Med. Sci., No. 125, 1903.

very unequal size and density of stain are pathognomonic of myelocythæmia. In some cases the protoplasm is swollen, hyaline, or vacuolated. Nuclei surrounded by granules scattered widely through the plasma, the protoplasm evidently having disappeared, are very common pictures. In the nuclei, karyolysis, vacuolation and karyorrhexis are common; pycnosis perhaps less so. Degenerated leucocytes are always present in leukæmia.

In the diagnosis the point upon which emphasis was formerly laid to distinguish it from an extreme leucocytosis was the large number of white cells. This distinction does not in the least hold, since there are periods of leukæmia with a count normal or even subnormal, and yet during this time the formula may be that of leukæmia. The presence of myelocytes alone does not give the diagnosis, since in cases of extreme leucocytosis a few myelocytes will usually be present. These myelocytes, however, are usually about the size of the ordinary leucocyte, and are never the very large cells which occur in leukæmia; also eosinophiles and Mastzellen are not increased. The diagnosis is especially difficult in children. In some cases autopsy alone will decide it.

A recent case on first admission had 443,000 leucocytes; was readmitted in fourteen months with a count of 9700; the count remained low till his discharge twenty days later with 100,000. On the day with the lowest count, 6000, the differential was: s. m., 3.8 per cent.; l. m. and tr., 3.6 per cent.; pm. n., 70.8 per cent.; eos., 3.8 per cent.; neutroph. myeloc., 8 per cent.; Mastzellen, 7.6 per cent.; normoblasts, 23; intermediates, 15; megaloblasts, 5 (per 1000 leucocytes). Hence even at this time a diagnosis could have been made.

Variations in the count are extreme over long periods of time; the daily variations are sometimes considerable, as in one case reported, with 122,500 at ten A.M., at four P.M. on the same day the count was 235,000.

With improvement in the condition the count may drop almost to normal. At this time the formula should help in the diagnosis, and yet this is not always true, since in some cases the characteristic blood picture has entirely disappeared. During such a period it would be impossible to differentiate a case with low red count from one of pernicious anæmia by the blood alone; and, indeed, there are cases reported of a transformation to pernicious anæmia and *vice versa.* Following the long-continued use of arsenic the count drops in a remarkable way, to rise at once after the drug is discontinued. Türk mentions a case [74] in which the leucocytes ranged from 258,000 to 370,000. After arsenic treatment they fell to 3000 to 6000 (0.5 per cent. myelocytes, 6.6 per cent. Mastzellen). It is a question how much improvement this indicates, as it may be an "exhaustion" of the bone-

[74] Deut. med. Wochenschr., 1904, No. 50.

marrow. Following X-ray treatment remarkable drops have been reported, even from 693,000 to 6300; the leukæmic character, however, was never lost. The red cells rose to nearly double in this case (Joachin and Kurpjuweit).

The infectious diseases which are survived, and this is rare, have a remarkable effect not only on the blood picture but also upon the blood-forming organs. This is particularly true of typhoid fever, influenza, miliary tuberculosis, *et al.* Chronic tuberculosis has very little influence. In Dock's case,[75] due to grip, the cells fell from 367,000 to 5000, then in six weeks returned to 157,000, and in one year to 461,000. The fall is sometimes extreme, as from 40,000 to 470. Some cases preserve the leukæmic formula, others do not. During the acute infection, when the count falls almost to normal, there is also a remarkable reduction in the size of the blood-building organs, and both sometimes return to their former condition in a few days after the infection is past; but not always, as in a case reported by McCrae; and others are on record in which at autopsy all signs of leukæmia had disappeared from the bone-marrow. In other cases, however, there is a rise instead of a fall, as in Müller's case of sepsis the leucocytes dropped from 246,900 to 57,300, then rose; in v. Limbeck's case of pneumonia they fell from 140,000 to 43,500, and then, as the other lung became involved, rose to 172,000. As the count drops the percentage of polymorphonuclears rises, the picture thus approaching that of a leucocytosis.

Late in the disease there may be a marked predominance of the large non-granular leucocytes, and there is good reason for the opinion that some of these are myelocytes without granulation, as if the body had lost its power to form the neutrophile material (Ehrlich).

Platelets.—In this form of leukæmia the platelets are markedly increased, even reach their maximum.

The *water content* is increased to 81 to 88 per cent.

The *specific gravity* is low, even 1036; that of the plasma about normal.

The *alkalinity* is somewhat decreased by the organic acids formed from the breaking down of leucocytes. Formic, acetic, lactic, and succinic acids have been found in the plasma. The xanthin bodies of the plasma are increased. Deutero-albumoses have been found. These are not present in lymphatic leukæmia, and are supposed to be digestive products of the leucocytes from a ferment provided by the polymorphonuclear cells. Taylor says that the nitrogen of the leucocytes is almost double. Nucleo-albumin is found in the serum, and uric acid, 22.6 mg. per 100 cc. Coagulation is increased, in one case so much so that the red blood-cells could not be counted with a pipette.

Lymphatic Leukæmia (Lymphæmia) (Plate II, A, B, C).—In this form of leukæmia is a marked increase of the mononuclear non-

[75] Am. Jour. Med. Sci., 1904, vol. cxxvii., a very exhaustive study of this subject, with a review of 50 cases.

granular cells. Despite the name, these cells are not all lymphocytes even in morphology, to say nothing of origin, but are mononuclear, non-granular cells of many sizes and forms. As a rule, while a variety of such cells are present, there is usually a predominance of one particular size; in some cases, the small mononuclear cells with a very narrow ragged rim of protoplasm; in others the cells are all of the large lymphatic type; in other cases the majority of the cells resemble the transparents, and in still others the transitionals of Uskow. In some cases the protoplasm of these large cells is basophilic; in other cases it is distinctly acidophilic; sometimes enormous cells are found.

As a rule there is a proliferation of the lymphatic tissue of the body, and yet not always, as is seen in some cases in which no lymph-glands are enlarged. In some of these cases there may be considerable proliferation of the large masses along the intestines; in still other cases the lesion seems limited to the bone-marrow. Some interesting cases begin with a great enlargement of the lymph-glands, the blood picture appearing later and coincident with it a diminution in size of these glands. Such was a recent case, who in September showed normal blood and a general enlargement of the lymph-glands. The following January he was readmitted with a leucocyte count of 110,000, chiefly the small cell variety. It is possible that the leukæmia does not begin till the disease of the lymph-glands has reached the marrow (Pappenheim).

Red Blood-Cells.—There is a much greater anæmia in this form than in the splenomyelogenous, and yet here also the count may remain normal for some time; later, however, cachexia with anæmia begins. In two of the chronic cases the count remained above 4,000,000 during a long stay in the hospital and until death. A remarkable case is reported (verbally) by Dr. Hazen, of eighteen months' duration, with reds 960,000, leucocytes 250,000, nearly all of the small lymphocyte variety. Cabot's average on first admission was 2,730,000; Osler's, 2,294,000; the average given by Hirz and Labbé is 1,829,000; by Petit and Weil, 1,292,000. The greatest reduction occurs in cases which show at autopsy the bone-marrow most involved, and in the acute cases, and the more acute the case the less the glandular enlargement. Nucleated reds are rare, sometimes absent, yet in the very severe cases there may be as many as in the splenomyelogenous variety. In one of our cases, with a total white count of 12,000, there were 150 normoblasts, 169 intermediates, and 20 megaloblasts per 1000 leucocytes,—a typical megaloblastic crisis. V. Limbeck describes them as astonishingly scarce.

The red counts remain very constant, often despite great fluctuations in the white cells (*e.g.*, 2,640,000 red cells, 105,000 leucocytes; in nine days 2,750,000

reds and 328,800; two weeks later, 2,892,000 reds, 410.000 whites; and again in two days, 2,928,000 and 480,000 whites).

In a case with pleural and ascitic fluids (chylous) repeatedly tapped, profuse diarrhœa, and death from streptococcus septicæmia, the white cells showed very slight variations, 133,400 at admission rising to 242,000, and at death 133,000; the red cells often above 5,000,000, from 4,912,000 on admission to 5,340,000 at death.

The **hæmoglobin** is diminished, Osler's average being 37 per cent.

The **leucocytes** are increased, on an average to 144,800 (Osler) or 141,000 (Cabot). In this form also there may be aleukæmic periods, which may last even six months. Just before death the count usually rises. One of our cases during the last ten weeks before death had a leucopenia of even 1900 cells. The count may be as high as in the myelogenous form, but this is rare.

Differential Count.—Grawitz classifies the cases, as those in which the increase is especially of the small mononuclears; those with an increase of medium-sized cells with basophilic homogeneous protoplasm; those in which the cells which predominate are very large and, for the most part, degenerated. Yet all these forms may occur together in varying percentage, and vary in the same case at different stages, for the case may start as a large-celled form and end as a small. Roser thinks that in cases in which the lymph-glands are particularly involved it is the smaller cells which are increased, *e.g.*, 99 per cent. of 117,000; and in those in which the lesion is particularly of the bone-marrow, the larger cells. Grawitz mentions an increase of the larger cells as simultaneous with a decrease in size of the lymph-gland. These mononuclear cells are often 90 per cent. of the total number, even 98 per cent., and in one of Osler's cases 99 per cent. A marked feature of the blood picture is the degeneration of these leucocytes, even 10 per cent. and antemortem even 75 per cent. showing some sign of degeneration, either of the protoplasm, or pycnosis and fragmentation of the nucleus. It is interesting that so few cells in the blood show mitotic figures, while in the bone-marrow the proliferation is very active. Many lymphocytes are very small, often even smaller than red blood-cells. They stain faintly in Ehrlich's stain, deeply in methylene blue; the protoplasm is scarcely seen, or is ragged and degenerated, the nuclei round or indented, and even fragmented, with a sharp margin and containing clear areas. Wolff thinks we should separate lymphatic from lymphoid leukæmia, the former being of lymph-gland origin, the latter myelogenous.

Polymorphonuclears are rare. *Eosinophile* cells are noticeably absent. In a pure case *myelocytes* are not present, although it is hardly wise to call the case mixed leukæmia if one be found. *Mastzellen* are absent, as a rule. In this form of leukæmia also the result of an acute infection is a drop of the total count, a true leucocytosis may supersede, and autopsy show the leukæmia cured. As a rule, even when the

count is low, the mononuclear cells will be 90 per cent. Wende's case [76] is a good illustration. The result of a streptococcus infection was a drop from 45,000 to 1600 leucocytes, but of small mononuclears from 95.3 per cent. to 88 per cent. The result of an acute infection is sometimes the appearance of a few myelocytes. Other cases show a marked increase in the count, as in Müller's case of chronic septicæmia it rose from 180,000 to 400,000.

In one case in this clinic the man was admitted with double tertian malaria and a lymphatic leukæmia of 105,000 (small monos., 83.6 per cent.; large monos. and tr., 7 per cent.; pm. n., 5.8 per cent.; eosinoph., 0.2 per cent.). One week after the malaria was cured the count rose to 328,000 and two weeks later to 480,000, with 97.2 per cent. small mononuclears. At this time there were 3 normoblasts, 3 intermediates, and 4 megaloblasts per 1000 leucocytes.

V. Limbeck considers that the blood picture alone is not enough for a diagnosis, since in some cases of sarcoma the blood presents a similar picture.

Acute Leukæmia.—This form of leukæmia, which has of late attracted a great deal of attention, is characterized by the brief course,—from six days to nine weeks (leukæmia acuta et acutissima),—the severity of the symptoms, the frequency of the hemorrhagic diathesis, the rapidly developing cachexia, and death. It occurs chiefly in young persons. The great majority of the cases are of the lymphatic type, but a few of the myelogenous variety have recently been reported; other cases are best described as mixed. The cases of the acute myelogenous form are collected by Gardinier,[77] who reports one and reviews eleven others, and also two doubtful cases (see also Billings and Capp).[78]

In all cases the anæmia is extreme, even below 1,000,000 red cells, and in Arneth's case 256,000 reds and 10 per cent. hæmoglobin.

The cases of acute lymphatic leukæmia are well reviewed by Rosenburger,[79] and acute leukæmia in children especially by Churchill,[80] who reports one case and reviews 28 others. The disease occurs even in the new-born child. The lowest red count was 750,000, after a severe hemorrhage. The leucocytes varied from 6000 to 810,000 (in a twenty-month-old child). The lowest counts came always just before death, which a falling count portends. Of these 29 acute cases in children, 25 were lymphatic (2 of the small-celled type, 3 large, 1 mixed); myelogenous, 2 mixed, 1 uncertain. Churchill's case had 99 per cent. small mononuclears, and many of them degenerated. The

[76] Am. Jour. Med. Sci., vol. cxxii., 1901.
[77] Johns Hopkins Hosp. Bull., October, 1904.
[78] Am. Jour. Med. Sci., 1903.
[79] Ibid., 1904, vol. cxxviii. p. 583.
[80] Ibid., 1904, vol. cxxviii.

anæmia is profound. It is of interest that the more acute the case the less the enlargement of lymph-glands and spleen. A good illustration is Pfannkuch's case, which ended fatally in three days with reds 2,500,-000; leucocytes, 1,000,000 (s. monos., 76.5 per cent.; neutrophilic myelocytes, 10.6 per cent.; neutrophile leucocytes, 12.2 per cent.).

Türk's case is a good illustration of the myelogenous form; red cells, 1,060,000; hæmoglobin, 19 per cent.; leucocytes, 42,000 (s. monos., 14 per cent.; pmn. n., 32 per cent.; myelocytes, 47 per cent.). The list of blood conditions somewhat similar to the acute myelogenous leukæmia are: an acute exacerbation of a chronic myelogenous leukæmia; a lymphatic leukæmia with an acute infection; an acute lymphatic leukæmia of the large-celled variety (since it is not easy to separate these cells from myelocytes); an acute infection causing grave anæmia, in which case there may be even 14 per cent. of myelocytes; an acute exacerbation of pernicious anæmia; malignant disease of bone-marrow, but the nucleated reds would suggest this last condition (Billings and Capps).

Some of these cases may be a sudden acute fatal exacerbation of a hitherto unsuspected leukæmia. Wey reported a case of the chronic myelogenous form which suddenly became acute, and only then large non-granular mononuclears appeared. In others the picture is that of an acute infection, and very likely soon such cases will not be considered as leukæmia. Clinically it is the anæmia that attracts attention, and the relation between these cases and pernicious anæmia is very interesting.

There is no type of cell characteristic of this form; Fränkel's unripe cell occurs commonly, but by no means exclusively here; in 3 of McCrae's 5 cases the small lymphocytes predominated. The cells are much more uniform than in the chronic forms, yet some are cases with large cells, some with medium, and some with small. In some nearly all the cells have a basophile protoplasm; in others it is acidophilic (Plate II, C).

The *red cells* show few changes; as a rule nucleated reds are scarce, yet in Herrick's case were 1800 per cubic millimetre, of which some were megaloblasts (but see McCrae's case). The drop in count showing rapid blood destruction is a striking feature.

But in some cases the leucocyte count is not high or even above normal; then for diagnosis the lymphocytes must be always high (Klein). Such cases at first resemble pernicious anæmia.

The cases of acute leukæmia in this clinic have been reported by McCrae.[81] They numbered five, all of the lymphatic type. Their average duration was six weeks, it varying from twelve days to eight weeks.

On admission, average of hæmoglobin, 35.4 per cent.; of reds, 1,822,000; of

[81] Brit. Med. Jour., February 25, 1905.

leucocytes, 104,000; highest, 326,000 (hæmoglobin, 45 per cent.; reds, 3,000,000); lowest, 57,800 (reds, 748,000; hæmoglobin, 16 per cent.). In one case, in fifteen days the reds fell from 3,000,000 to 1,450,000. Color-index is high, 0.93 to 1.4. The small mononuclears varied from 94.2 to 99.4 per cent., and in three the small lymphocytes were the prevailing cell, unusual in this form of leukæmia. In one case occurred many large lymphocytes which were actively motile. Nucleated reds were absent in two cases, 2 per 1000 leucocytes were found in three cases and in the fifth case 310 per 1000 leucocytes; *i.e.*, 3720 per cubic millimetre, of which 7 per cent. were megaloblasts and 48 per cent. intermediates. McCrae emphasizes the high color-index of these and of the cases in literature, and finds that of 45 cases in 24 the red count was below 1,500,000, and in 38 of the 45 below 2,500,000. Of 40 cases from literature, in 20 the color-index was 1 or above, and in 20, under 1. The low count and high index of the primary anæmias seem to him a special feature of this type of leukæmia. A most remarkable case was recently admitted with reds, 752,000; hæmoglobin, 17 per cent.; leucocytes, 880,000, large-cell type.

Of 13 cases of acute leukæmia in children, collected by McCrae,[82] the anæmia was severe (highest red count, 2,350,000; lowest, 1,000,000), color-index high; the red cells were of normal appearance; no nucleated reds were found in 7 cases, few in 4, and megaloblasts in but one.

The leucocytes varied from 21,000 to 209,000; there was no relation between acuteness and character of cells. The absolute number of polymorphonuclears was about normal in all cases. In these cases the anæmia is the important feature; the leukæmia would not have been suspected without blood examination.

Mixed Leukæmia.—These cases may best be described as a lymphatic leukæmia with a considerable number of myelocytes, both eosinophile and neutrophile. A few myelocytes may occur in lymphatic leukæmia, and the question is one of drawing a line.

The **cause of leukæmia** is still to be discovered. A few words, however, may be said concerning Löwit's organism. In the splenomyelogenous form he described

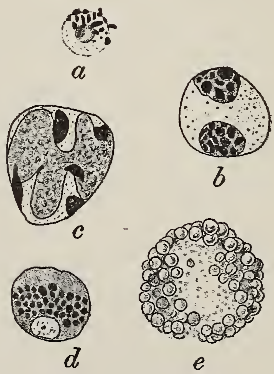


FIG. 115.—*a*, *b*, *c*, *d*, four leucocytes containing Löwit's organisms (copied from Löwit); *e*, large granular (and vacuolated?) cell of bone-marrow.

Hæmamœba leukæmiæ magna; specific bodies occurring in the large and small mononuclears, both non-granular and granular, rarely in the polymorphonuclear cells, never in the reds, sometimes free in the plasma. Their size varies much, and sometimes all varieties in the same cell. This he considers is due to multiple infection, while some of the groups of small cells he considers evidence of multiplication. They are in or on the leucocytes, often in vacuoles or splits of the

[82] Johns Hopkins Hosp. Bull., May, 1900.

protoplasm. They look as if they were amœboid when they died. They can be stained by a particular method, but they differ much in their staining qualities. Navicular bodies or crescent bodies are found which first suggested to Löwit the parasitic nature of the disease, since they resembled similar bodies in the coccidia and the hæmosporidia. These were never found in other conditions. He considers that he can exclude artefacts. He found them in the blood-building organs, and states that he got positive results from rabbit inoculation.

In the lymphatic form he describes Hæmamœba leukæmiæ parva. This he found, however, in but one of five cases studied. They are smaller than the preceding, seem more amœboid, are found especially in the blood-building organs, very rarely in the peripheral blood, points of resemblance analogous to the æstivo-autumnal malaria parasite. Those forms which he considers segmenters have a different arrangement and fewer segments. In this form no resting stage was found, and he is not certain of navicular bodies.

These parasites of Löwit have been the subject of a very animated discussion, particularly between himself and Türk who considers that they are much altered basophile granules or artefacts. The technic to demonstrate them is difficult and his findings unsatisfactory. We would expect the protozoa, if such they be, to show, as do others of a somewhat similar class, a little more uniformity in size, and a life history analogous to theirs. Studying Löwit's plates it would seem difficult to bring order out of such a chaos of forms. By unanimous consent the question has been allowed to drop, and yet in the blood specimens we have seen stained with his method even if all were artefacts they were beautiful pseudoparasites.

Pseudoleukæmia.—Under this heading have been grouped a great variety of diseases, including Hodgkin's disease, which now, by removing a superficial gland, can be easily excluded; tuberculosis of the lymph-glands, which can be recognized by the tuberculin test; lymphosarcoma and malignant lymphomata, which the pathologists say can be recognized anatomically. Others include splenic anæmia. Hence scarcely anything is left in the group of pseudoleukæmia, and it is the opinion of some that a "true" pseudoleukæmia is still to be proved (Reed); that is, a disease with the anatomical features of leukæmia, but not its blood-changes.

By the term pseudoleukæmia is generally meant a condition the clinical picture of which (the swollen lymph-glands and the cachexia) is that of lymphatic leukæmia, but without its blood-changes, and such cases are common enough, as the above list shows. Some cases which could be brought under this head are certainly early stages of lymphatic leukæmia, or cases during aleukæmic periods, yet other cases are chronic, even for eight or ten years.

Hodgkin's Disease.—The blood features of this disease are those of cachexia. In the eight cases reported by Reed the red count varied from 3,232,000 to 5,264,000 on admission. One case was as low as 2,670,000, but afterwards improved. These cases were, therefore, somewhat anæmic, and two showed a severe anæmia of the secondary type. At the onset the count may be practically normal and for months remain so despite the rapid growth of the lymph-glands. Then begin the changes of an ordinary secondary anæmia, often extreme, with at the end a count as low as 1,522,000, with degeneration of the red cells,

nucleated reds, and poikilocytes which are noticeably rare except at the late stages. Leucocytes are slightly increased, averaging about 12,000. In two of the eight cases there was an absolute increase of small mononuclears, but the lymphocytosis of Pinkus is by no means constant. The maximum of the small mononuclears was 38.6 per cent., the absolute count, therefore, 5304, and the next highest 4600 (36.8 per cent.). In two cases the small mononuclears were low, in one 2 per cent., or 310 cells, in another 9.4 per cent., or 940 cells. Grawitz considers that the differential count is no aid in diagnosis, but that a slight increase does accompany improvement; a decrease, a decline.

Tuberculosis of the Lymph-Glands.—In cases of general glandular tuberculosis there may be a normal red and leucocyte count, in other cases a cachexia with a secondary anæmia. Very interesting cases have a considerably lowered count of leucocytes, even 300 cells per cubic millimetre.

Of this we have recently had 12 cases. Four of these showed a leucocytosis of 11,000 to 29,000. The red cells were slightly decreased (two cases, 3,600,000 and 3,700,000, and 6 cases from 4,000,000 to 5,000,000), the hæmoglobin was much reduced and in 6 cases the index below 0.6. As a rule, there is no leucocytosis until a secondary infection occurs.

A case like the following is a puzzle for diagnosis. The woman, aged fifty years, had a red count of 4,000,000; hæmoglobin, 50 per cent.; leucocytes, 8000. She had tuberculosis of the lungs and swollen lymph-glands, two of which were removed with an interval of one year, and both pronounced tuberculous. She had night-sweats and lost weight. Three months after the above blood-count the glands began to swell enormously; red cells, 3,000,000; leucocytes, 80,000, 96 per cent. of which were polymorphonuclear neutrophiles. A little later the count had risen to 120,000; lymph-glands and spleen enormous. She received X-ray treatment, and in three weeks the leucocytes were 16,000 and the reds 2,100,000. She died soon after.

Futcher reported a case with leucocytes 300 per cubic millimetre, and thinks the blood little aid in the diagnosis between tuberculosis of the lymph-glands and Hodgkin's disease except that it is more often normal in the latter.

Leukanæmia is the name given by v. Leube to a group of cases formerly grouped by some with the acute leukæmias, and by others with the pernicious anæmias; the features of both are present. Rather than as an independent blood disease it is considered [83] a symptom of a large number of conditions,—injuries, hemorrhage, intoxications, infections, malaria, malignant growths, etc. There is severe anæmia with nucleated red of all varieties, and a normal or increased white count, with many myelocytes, but no eosinophiles. Other cases resemble lymphatic leukæmia.

The anæmia usually precedes the increase in white cells.

BLOOD IN ACUTE DISEASES

Malaria.—The anæmia of malaria is important in diagnosis, since it is one of the earliest symptoms, the count dropping from 5,000,000 to

[83] Luce, Deut. Archiv. f. klin. Med., 1900, vol. lxxvii. p. 215.

even 500,000 in a few days. It is due both to the direct destruction of the corpuscles by the intracellular parasites and to the destruction of red blood-cells not containing parasites, the latter seen especially in those cases with hæmoglobinuria, in which the hæmolysis is serious.

The red blood-cells decrease after each paroxysm, slightly in the tertian and the quartan malaria, more in the æstivo-autumnal in which case there may be a drop of 1,000,000 cells after one paroxysm.

In 54 cases of æstivo-autumnal malaria the red cells were between 1,000,000 and 2,000,000 in two cases, 2,000,000 and 3,000,000 in 12, 3,000,000 and 4,000,000 in 20, and 4,000,000 and 5,000,000 in 12, above 5,000,000 in 8. In 56 cases of tertian the figures for these same limits were 1, 10, 28, 13, and 4 respectively. It is seen that in our cases these two forms differ little. The mean count for each was 3,500,000. But in this climate we seldom see truly pernicious cases.

In the "pernicious" cases the count drops somewhat also between the paroxysms. In Grassi's case there was a loss of 4,000,000 cells in six days. In one case the count at the end of thirty days was 500,000. Dionisi reports a case in which each chill cost from a half to one million red blood-cells. The greatest fall is in the earliest paroxysms, later less, until finally the count remains almost stationary despite repeated paroxysms. In cases with pernicious malaria and hæmoglobinæmia the anæmia is grave, with poikilocytes, endoglobular degenerations, occasional shadows, fairly numerous nucleated reds, increased platelets, and leucocytosis.

The regeneration of the cells in the tertian and quartan is rapid, the count often being restored before the next paroxysm, and anæmia occurring only after a long series of chills. In the æstivo-autumnal the recovery is slower, the new cells pale, varying in size and shape; nucleated reds are common, and since the anæmia is chronic the regeneration is slow, and grave anæmia may result. This slowness in regeneration is due also to the extensive necrosis and the resulting fibroid induration of the bone-marrow, which may be the chief seat of the infection, and to the accumulation of pigment in this tissue. The hæmoglobin suffers even more than the red blood-cells and returns to normal more slowly.

The leucocytes are almost always subnormal in malaria, an important point in diagnosis, except in the grave pernicious paroxysms. They rise slightly (to 6700, some say to a true leucocytosis) just before the paroxysm, and then steadily fall, reaching a minimum (average 2300) at the time the temperature is subnormal, and sometimes to as low as 1000 to 2000 cells.

In 82 recent cases of æstivo-autumnal malaria the leucocyte counts were, from 1000 to 2000, 3; 2000 to 5000, 8; 3000 to 4000, 21; 4000 to 5000, 15; 5000 to 6000, 14; 6000 to 7000, 8; 7000 to 8000, 4; 8000 to 9000, 2; 9000 to 10,000, 2; above 10,000, 5, one of these a pernicious case (14,500); mean 3500.

In 70 cases of tertian malaria the figures for these same limits were 2, 5, 11, 18, 10, 10, 5, 2, and 2; above 10,000, 5; highest, 16,500; mean, 4500.

The differential count shows a relative decrease of the polymorphonuclear neutrophiles, and an absolute increase of the large mononuclears. The mean averages found by Thayer are: Small mononuclears, 16.9; large mononuclears, 16.9; polymorphonuclear neutrophiles, 65; eosinophiles, 0.9 per cent.; in grave cases, myelocytes 2 to 3 per cent. (Cabot). The increase of the large mononuclears, very pronounced in the apyretic periods, is usually absent in the pyretic periods. If as the temperature falls these cells do not increase, it is evidence against malaria, while they are most valuable in the diagnosis of cases admitted after they have taken quinine, and hence without parasites in the peripheral blood. In the tropics Stevens and Christophers say that over 15 per cent. large mononuclears means an actual or a recent malaria; with 20 per cent. one almost always finds the parasite. This increase affects the cells which Uskow calls "transparents," a group of cells which vary in size from lymphocytes to the largest cells of the blood, which are slightly amœboid and distinctly phagocytic, in fact, the chief phagocytes in malaria, sometimes containing a few pigment granules, sometimes many, these pigmented cells being almost as important in diagnosis as is the parasite itself.

They occur much of the time in the æstivo-autumnal form but only after the paroxysm in tertian and quartan. These phagocytic cells are said to rapidly become necrotic and to disappear from the circulation, which explains the diminution in the count at the end of an attack.

Stephens and Christophers cite, as illustrations of this relation of the leucocytes, the following cases: Bastianelli's fatal comatose case of æstivo-autumnal malaria, in which the small mononuclears were 19.1 per cent., the large mononuclears and transitionals, 41 per cent.; polymorphonuclear neutrophiles, 39 per cent., and eosinophile cells, 0.6 per cent. Panse's case, with a temperature at 37.2° C., and the small mononuclears, 18.1 per cent.; large mononuclears and transitionals, 26.4; polymorphonuclear neutrophiles, 55.3. In another case with temperature normal the small mononuclears were 14.8 per cent.; large mononuclears and transitionals, 46.7 per cent.; neutrophiles, 38.5 per cent.

In one of our tertian cases the total leucocytes were 16,500; large mononuclears, 38.3 per cent.; in a case of æstivo-autumnal, leucocytes, 6000, large mononuclears, 26 per cent.; in another, 4000 and 22 per cent.

A leucocytosis occurs rarely except in pernicious paroxysms. In one case in this clinic the count one hour before death was 50,000, of which the large mononuclears and transitionals were 18 per cent., the

polymorphonuclear neutrophiles, 58 per cent. It occurs also in malarial hæmoglobinæmia, in which case there is a marked increase in the polymorphonuclear neutrophiles, which begins with the attack and lasts for some time. A leucocytosis is seen also during the death agony, and is due to complications. There is a definite leucocytosis in the post-malarial anæmia, sometimes with increased eosinophiles and with myelocytes.

Bignami and Dionisi classify the anæmias of malaria as follows: Those which follow ordinary acute malarial fever, in which there is a secondary anæmia of a chlorotic type, without leucocytosis, with a few nucleated reds, leucocytes reduced, the large mononuclears relatively increased; (2) cases resembling primary pernicious anæmia, usually fatal, with extreme oligocythæmia, marked poikilocytosis, high color-index, nucleated reds if present for the most part megaloblasts, leucocytes diminished, and lymphocytes relatively increased; (3) rapidly fatal cases without any signs of regeneration which may have been in the first stages those of simple secondary anæmia. This anæmia is very similar to that which follows a severe hemorrhage.

(4) Chronic grave secondary anæmias of a chlorotic type and without nucleated reds; leucocytes much reduced. This is seen in a chronic malarial cachexia, and is due for the most part to degenerative changes in bone-marrow occurring after long infections, with the marrow sclerotic and pigmented (Thayer).

Septicæmia.—That septicæmia does not always cause a leucocytosis is seen in typhoid fever and acute miliary tuberculosis, and yet as a rule it does, but less constantly and markedly than do local pus accumulations. In the streptococcus and staphylococcus septicæmias, however, there is often a marked anæmia, with a greater and earlier fall than in any other acute disease. In one case of acute streptococcus septicæmia with hemorrhages (Grawitz) the cells fell to 300,000 in twenty-four hours. Septic fevers as a rule cause a loss of from 200,000 to 1,000,000 cells per week; that of puerperal infection has an especially bad effect upon the reds. The qualitative changes are marked,—degeneration, poikilocytosis, polychromatophilia, etc.; nucleated reds occur seldom in large numbers. The leucocytes vary as the patient's resistance, in some cases being very high, in other cases even subnormal.

We have had 26 cases of well-marked septicæmia. The final red counts in 15 fatal cases were from 1,000,000 to 2,000,000 in 2; 2,000,000 to 3,000,000 in 3; 3,000,000 to 4,000,000 in 4; above 4,000,000 in 6.

It is thus seen that the toxine does not kill through the anæmia (the drop in count after admission and before death was from 900,000 to 1,600,000), and that there are two groups of cases, those with high and those with low counts.

In 4 cases there was no leucocytosis at death. In 21 cases the leucocytes varied from 11,000 to 47,000. Some cases showed great variations in the white count. In one case with 8000 leucocytes 96.6 per cent. were polymorphonuclears; in another, of 10,400, 92.6 per cent. Gonorrhœal septicæmia caused a drop to 2,318,000, with 30 per cent. hæmoglobin, and at death leucocytes, 47,000.

Chronic septicæmia it is important to diagnose, those cases of cryptic origin often passing unrecognized. In Ewing's case of em-

pyemia, with a duration of one year, the red blood-cells were 1,800,000, and the hæmoglobin 25 per cent. In other cases of pelvic abscess of even two years' duration the anæmia is slight.

We have had recently four cases of **Typhus Fever:**

Case I.—Male, thirty-six years; red cells, 5,400,000; hæmoglobin, 72 per cent.; leucocytes on admission, 18,600; temperature, 103 to 104° F. On the eighth day after admission, 25,400; the temperature had begun to fall. Five days later, temperature normal; total count, 24,300; s. m., 3.2 per cent.; l. m. and tr., 6.6 per cent.; pmn. n., 90 per cent.; eosinoph., 0.2 per cent.

Case II.—Male, nineteen years; red cells, 4,500,000; hæmoglobin, 70 per cent.; leucocytes on admission, 8600, and remained normal for four days; temperature, 102° to 105° F. On the fifth day, count 12,500; on the tenth day, with temperature normal, total, 10,000; s. m., 6 per cent.; l. m. and tr., 4.2 per cent.; pmn. n., 89.4 per cent.; eosinoph., 0.2 per cent.

Case III.—Male, thirty years; red cells, 5,500,000; hæmoglobin, 85 per cent.; leucocytes on admission, 7000, and remained normal three days; temperature, 101° to 103° F. On the fifth day, 38,000; temperature, 98° F.; death (total count, 38,000; s. m., 5.8 per cent.; l. m. and tr., 1.2 per cent.; pmn. n., 93 per cent.).

Case IV.—Male, twenty-two years; red cells, 5,400,000; hæmoglobin, 85 per cent.; leucocytes on admission, 9200, and normal three days; temperature, 102° to 104° F. On the tenth day they had risen to 11,600, but the temperature had already been normal three days. Leucocytes normal on the twelfth day (total, ninth day, 10,800; s. m., 15 per cent.; l. m. and tr., 11.5 per cent.; pmn. n., 72 per cent.; eosinophiles, 1.0; Matzellen, 0.8 per cent.).

From these four cases (daily counts were made in all) it is seen that the leucocytes are low on admission, even normal, then rise to a maximum, which occurs when the temperature has begun to fall or is already normal, then they fall to normal. (Compare with influenza and variola.)

This is different from the findings of Ewing and Thomas, who report absence of leucocytosis.

Measles and **German measles** have almost no influence on the red blood-cells, and cause no leucocytosis, or only a slight. In the post-febrile stage the large mononuclears are increased.

Plantenga claims, in the thirteen cases of measles and nine of Rötheln which he studied, a neutrophile hyperleucocytosis of even 20,000 during the prodromal stage, which rapidly gave place to a hypoleucocytosis during the eruptive stage, due to the disappearance of the neutrophile cells, and with sometimes a lymphocytosis and disappearance of eosinophiles.

Renaud found in six cases that this preliminary leucocytosis reached its maxi-

mum about six days before the rash had appeared. This leucocytosis permits one to isolate a suspected case early.

Tileston could not confirm this leucocytosis during the prodromal stage, and thought all leucocytoses could be attributed to a complication.

We have very little material, but in nine recent cases in only one was the count above 8600 during the height of the fever (17,200).

Scarlet fever has very little effect upon the red blood-cells, but does cause a slight anæmia, the count averaging 4,500,000 (Reckzeh). The leucocytes are uniformly increased, an important point in diagnosis, rising during the incubation period, even six days before the rash, and continuing elevated into convalescence and even twelve days after the temperature has reached normal, an interesting exception to the rule of other diseases that the count is roughly parallel to the temperature. They are generally normal on the fourteenth day (Reckzeh). The leucocytes vary from about 10,000 to 40,000; mild cases, 10,000 to 20,000; moderate, 20,000 to 30,000; severe, 30,000 to 40,000, according to the severity of the case and its duration. The neutrophile cells are relatively increased (to 85 to 98 per cent., especially in fatal cases); the eosinophile cells rapidly disappear and reappear with improvement; their failure to reappear is considered a bad sign. As a rule they reach normal after the leucocytosis has disappeared; their maximum is two or three days after the rash appears. This early presence of eosinophilia is important in the diagnosis, excluding various septic conditions.

Diphtheria.—In this disease there is a moderate anæmia, a loss of about two million cells at the time of defervescence. During the height of the disease, however, there is often an increase in the count and specific gravity. (The injection of bacilli or their toxines into the circulation of animals causes a lymphagogue action which results in a hypercythæmia.) This hypercythæmia occurs most commonly in this of all the acute infections. In the case of Cutter the cells were from 7,200,000 to 7,800,000; in that of Morse from 5,000,000 to 5,500,000 during the first week, and 6,800,000 during the second. With the drop in the count the nucleated reds and the polychromatophilic cells appear. There is a slight leucocytosis—as a rule, 10,000 to 15,000, but in severe cases even 17,000, and with complications 30,000—which varies as the severity of the infection. The leucocyte curve runs parallel to the temperature, and its degree is roughly parallel to the amount of false membrane. The rise is in the polymorphonuclear cells. In some fatal cases there is leucopenia. The myelocytes are increased, especially in the fatal cases, to from 3 to 16 per cent.

In ordinary follicular tonsillitis the counts are often as high as in diphtheria.

Smallpox.—"No other disease is so destructive to the red blood-cells" (Hayem). The anæmia is evident as the temperature falls, and

the question often arises whether it is truly due to a destruction of cells or merely to a dilution of the blood from the relaxed vasomotor tone. Yet during the pustular stage especially there may be a loss of 2,000,-000 cells. Regeneration is slow, lasting about fourteen days. In the hemorrhagic form the anæmia is severe and varies with the amount of hemorrhage. Pick and Weil say that there is anæmia in the severe, none in the mild cases. Malassey says that the drop begins in the pustular stage, and that the rise begins with the desiccation or even during convalescence. The nucleated reds (normoblasts) are rare, except in the hemorrhagic form, in which they may be very numerous.

Leucocytes.—From the onset with a normal count the blood formula is very characteristic of this disease. The polymorphonuclear neutrophiles are decreased, averaging about 40 per cent. or even 20 or 14 per cent.; the small mononuclears vary from 30 to 40 per cent.; the large mononuclears from 4 to 10 per cent.; myelocytes and irritation forms each 2 to 10 per cent.

The disease *per se* causes no leucocytosis, but during the pustular stage the leucocytosis is said to be the result of the infection by the skin cocci.

Tuberculosis.—Tuberculosis is a disease the virus of which can cause anæmia of the highest grade (*e.g.,* v. Limbeck's case of tuberculosis of the peritoneum and other abdominal organs, with a red blood-count of 730,000 and hæmoglobin 25 per cent.; such cases are so rare that this one is doubted by Cabot), but usually one of moderate grade, one often more apparent than real, and which may not exist at all. The degree of the anæmia is independent of the localization of the disease.

In tuberculosis in general a mild grade of chlorotic anæmia is the rule. Such occurs in slight involvement of the apex ("anæmia of onset") without fever, in tuberculosis of bones and lymph-glands. This is the "pseudochlorosis tuberculosa." The count is almost normal, the leucocytes normal, the hæmoglobin somewhat reduced. In other cases there is a lymphocytosis, absolute or relative; in some few cases a reduction of the count as well as of the hæmoglobin. Qualitatively some of the red blood-cells (not the majority, as in chlorosis) are seen to be pale and small; poikilocytes are usually few, may be extreme, but are rarer than in other cachexias of the same degree; nucleated reds are rare, even after a severe hemorrhage and when the anæmia is extreme, a point of importance in the differential diagnosis between this and carcinoma; Maragliano's endoglobular degeneration is seen in severe cases, especially in those of mixed infections.

Cabot considers that the tuberculous virus has itself but little effect on the blood, and that the above-mentioned changes are due to secondary infections, or to drains upon the proteid of the blood from diarrhœa, effusions, starvation, prolonged suppuration, etc.

In a pure case, with the exception perhaps of meningitis, the leucocytes are not affected. This is important in the diagnosis of peritonitis, bone troubles, and acute miliary tuberculosis. Qualitatively in some cases there is no change, and yet in others with a normal count is seen the increased percentage of the mononuclear cells common in all conditions with poor nutrition. If there is a leucocytosis, it is of the ordinary inflammatory type. The eosinophile cells are increased in some cases with cavity formations, and since this occurs also after the injection of tuberculin some think it is due to an auto-intoxication from the cavity. Myelocytes appear in advanced cases.

Chronic Tuberculosis of the Lung.—Grawitz has divided these cases into three groups: Group 1, with slight involvement of the apex, without fever, showing clear signs of anæmia, the pseudochlorosis tuberculosa, some with a normal count, others with a slight reduction, the leucocytes normal. Some early cases have almost normal blood. Whether there is a group in which the blood signs precede the physical signs may depend on the care with which the physical signs are sought. Group 2, cases of chronic phthisis with cavity formation but without other complications, the temperature slight; the blood picture is remarkably normal as regards count, hæmoglobin, specific gravity, dry constituents, yet there is with the cavity formation a general emaciation. Such a patient earlier had a distinct chlorosis; the leucocytes are normal or slightly increased, from 10,000 to 15,000 per cubic millimetre. Group 3, of cases with hectic fever, supposed by some to be due to a secondary infection but by others to pure infection with the tubercle bacillus. In these cases there is a true anæmia, a diminution in the count, sometimes rapid, of the hæmoglobin and dried substances, an anæmia which progresses often until death, with evidence of true blood destruction. The drop in the count may even be rapid.

In a recent case of pulmonary tuberculosis, two days before death the red cells were 1,473,000; hæmoglobin, 15 per cent.; leucocytes, 9000 (pmn. n., 88 per cent.; s. m. 5.9 per cent.; l. m. and tr., 4.7 per cent.; eos., 0.35 per cent.; normoblasts, 4, megaloblasts, 4 per 1000 leucocytes)

There is leucocytosis as a rule, especially if there be a secondary infection of which v. Limbeck considers it sufficient guarantee, and which is important in the diagnosis. In the chronic septicæmia form is a slight, and in caseous pneumonia as high a leucocytosis as in croupous pneumonia.

The normal count in the second stage has aroused considerable discussion. A concentration of blood by sweating, diarrhœa, and vomiting is not alone sufficient, although, if present, will help. Some consider the hypercythæmia a compensatory feature for the dyspnœa, since dyspnœa due to any cause produces a hypercythæmia;

others admit an anæmia which is covered by an oligæmia, and autopsies at this stage suggest a diminution of the total volume of blood. V. Limbeck considers the changed water metabolism the important point, the general drying of the tissues concentrating the blood; *i.e.*, an oligæmia vera. Grawitz considers the absorbed products of caseous nodules to have a lymphagogue effect, thus concentrating the blood.

After hæmoptysis the regeneration may be rapid (see page 517); after operation on a tuberculous focus if the hæmoglobin does not return rapidly the operation was probably incomplete; in some cases the anæmia accompanies the dissemination of the bacilli; in others the count rises and one can find no parallelism between the general condition of the patient and his blood; in fibroid phthisis, as a rule, there is no leucocytosis; in acute phthisis the anæmia is pronounced and progressive. In cases of cavity formation there is almost always a leucocytosis. In extensive tuberculous pneumonia some have little, others as high a leucocytosis as in croupous pneumonia. Acute miliary tuberculosis presents no change in the red blood-cells or hæmoglobin, and the leucocytes usually remain normal, but in a few cases are very low, even from 500 to 600 cells, over 90 per cent. of which are polymorphonuclear neutrophiles.

Tuberculosis of the serous membranes is accompanied by a mild secondary anæmia without leucocytosis unless the blood be concentrated by diarrhœa, except, perhaps, in some cases of meningitis, which is accompanied by a leucocytosis (Osler). In tuberculosis of the glands there is no leucocytosis until caseation begins. The injection of tuberculin into a tuberculous patient causes a leucocytosis with a rise of eosinophiles. In tuberculosis of the bones there is a marked absence of leucocytosis until a secondary infection sets in; a high count indicates abscess formation, but after the abscess has become chronic the count may remain normal until a secondary infection occurs. In these bone cases the reds are rarely diminished, but the hæmoglobin is low.

The very slight anæmia found in children is rather remarkable, since their blood is usually so susceptible. Brown,[84] in 73 cases, found the red blood-cells diminished only in the long-standing extensive cases in very young persons, but the hæmoglobin was diminished somewhat in all.

Of 17 cases of ACUTE MILIARY TUBERCULOSIS, in 5 cases the red cells stood between 3,600,000 and 4,000,000, and in 6 over 5,000,000. The color-index was quite low, in one-half the cases from 0.4 to 0.6. The leucocytes varied from 1000 to 9000, the majority (9) from 3000 to 6000.

One case had an interesting differential count (total, 3500; s. m., 6.5 per cent.; l. m. and tr., 10.8 per cent.; pmn. n., 81.9 per cent.; eosinophiles, 0.5 per cent.). Whartin's case with lower count, had 91.48 per cent. pmn. n.

Whartin reported a case with leucocytes often below 2000, and on one day (with a chill) 600, and Cabot a case with 550.

[84] Trans. Med. Soc. of the State of California, 1897, p. 168.

Tuberculous meningitis is an illustration of the general rule that the effect of an infection upon the leucocyte count depends partly on the location of the lesion, for tuberculosis of the meninges usually causes a leucocytosis.

Of 15 cases, in only 3 was the count below 10,000, and one of these was only a part of an acute general miliary infection. In the other two, one count each was made. The leucocyte curve is a very irregular one, 3 of our cases with high counts showing periods with low counts.

The highest count was 26,800.

In the series of 43 cases reported by Cabot there was leucocytosis in 32.

Tuberculous Peritonitis.—During the past four years we have had 22 cases. Of 19 cases in which the red cells were counted, 7 were between 3,000,000 and 4,000,000, and 6 between 4,000,000 and 5,000,000. The color-index varied from 0.5 to 1. Of the 22 cases there was a leucocytosis in 9 (highest count, 22,400).

Of Cabot's 60 cases there was a leucocytosis in 14.

Tuberculosis of Bones and Joints.—These cases are reported chiefly from surgical clinics. There have recently been 15 cases in this clinic, with a leucocytosis in 6.

It is believed that during the active process of abscess formation there is a leucocytosis which in time will disappear, to reäppear in greater degree if a secondary infection occurs; hence the high jump in the count which is a measure of the sepsis following operation, as a rule, this leucocytosis will soon subside, and so long as the abscess drains freely either not appear or the count will remain fairly low.

Tuberculosis of the Intestine.—Of 5 cases there was a secondary anæmia in 2 (3,300,000 and 2,800,000 red cells). In one a leucocytosis of 14,000, which disappeared soon after admission.

Two cases of renal tuberculosis showed no leucocytosis.

One case of Addison's disease had red cells, 6,600,000; hæmoglobin, 92 per cent.; leucocytes, 9000. Sometimes there is marked anæmia in this disease.

Typhoid Fever. Red Blood-Cells.—In the fresh blood smear may sometimes be found the very large phagocytic cells crowded with red blood-cells which Mallory has emphasized. Some find a slight rise of reds during the first week, then a slow fall to normal, and at the time of defervescence a true drop. Thayer[85] in the cases of this clinic found at the end of the first week a count of about 5,000,000 red cells, and then a gradual reduction until defervescence, when, as a rule, regeneration begins. In cases with very long-continued fever the regeneration may begin slightly before the temperature is normal. The loss of reds averages 1,000,000 cells. The end of the third week is the average limit of the disease, at which time the average count of our cases was 4,555,814. The fall may be accentuated during the fourth week, and, indeed, the usual statement is that the anæmia begins at this time. During this period there are transitory variations in the count due to vomiting, sweating, diarrhœa, etc. Following a severe hemorrhage the anæmia is manifest, and regeneration begins at once.

Following some very severe cases is a post-typhoid anæmia, in one case with 1,426,000 red cells during the fourth week; in another

[85] Johns Hopkins Hosp. Rep., vol. viii.

case 1,300,000 during the third week (both Osler's cases), and one case of 804,000 (Henry). Usually there are no qualitative changes. After a hemorrhage are sometimes seen nucleated reds.

There is always a more marked reduction in the HÆMOGLOBIN than in the reds, the color-index, according to Thayer, varying from 0.7 to 0.8. In the above case with 1,300,000 cells the hæmoglobin was 20 per cent. The hæmoglobin runs parallel to the red blood-cells, but returns to normal more slowly.

The LEUCOCYTES, some think, are slightly increased at the very first, but apart from this they are subnormal during the whole course, and gradually diminish from the first (with 6400) to the fifth week, at which time the average of our cases was 5386. Some cases reach 2000, 1000 per cubic millimetre, or even lower. Thayer found no cases with an initial leucocytosis. The longer and more intense the infection the lower the leucocytes. In still other cases the count is above 10,000 throughout the whole course, cases without any complication. There may be temporary variations, the count rising to 10,000 cells after a cold bath, *e.g.*, yet with the differential count unchanged.

The *differential count* for the first five weeks shows a decrease in the percentage of polymorphonuclear neutrophiles, usually to 60 per cent., and below 50 per cent. not rarely, and an increase of the mononuclears, especially the large, the transparent cells of Uskow, cells which are morphologically not lymphocytes, but which vary in size from these to the largest cells of the blood. They have relatively abundant protoplasm and faintly staining nuclei. These are especially numerous at the height of the fever. The eosinophile cells are reduced below 1 per cent., as a rule, until convalescence, when they increase to even above normal. They may, however, in long continued cases, increase with the increase in reds before the temperature is normal. During convalescence the count returns slowly to normal, but the blood retains its characteristic features for about three weeks after the temperature is normal.

The blood picture is modified by VARIOUS COMPLICATIONS. Hemorrhage causes an acute post-hemorrhagic anæmia with leucocytosis, the lowest count of our series being 1,992,000 cells; regeneration begins at once, and the cells are usually restored in a little over one week.

The inflammatory complications are accompanied by a rise, or even a true leucocytosis. This is true of furunculosis, phlebitis, thrombosis, bronchitis, periostitis, pleurisy, pneumonia, etc. A definite rise in the count, already very much reduced, is for that person often a true leucocytosis; for instance, in one case in our wards the leucocyte count accompanying parotitis rose from 1600 to 3200 cells, comparable to a rise in a normal person to about 15,000 cells.

In one case of empyema the count was 44,500; in a second case, due

to the typhoid bacillus, 23,000 cells, of which 68.5 per cent. were polymorphonuclear neutrophiles; small mononuclears, 12.7 per cent., and large mononuclears, 17 per cent.

Of the 5 cases of pneumonia of our series, 3 cases, of whom 2 died, had counts above 10,000 cells; 2 cases, both of whom died, below 10,000 cells. In all of these cases the differential count showed a smaller percentage of the polymorphonuclear neutrophiles than one would expect. In periostitis due to the typhoid bacillus the leucocytes in one case were 18,000, of which 72.5 per cent. were polymorphonuclear neutrophiles. Thayer cites many similar illustrations showing that in an inflammation due to the typhoid bacillus the reaction of the blood depends more upon the situation of the infection than upon the organism, and the tendency, as illustrated by the above cases of empyema and periostitis, is for the formula of typhoid fever to persist.

Perforation.—In our cases of suspected perforation the leucocytes are followed with the greatest care, and interpreted in general as in appendicitis. From the onset of the first symptom, whether it be abdominal pain, hiccough, or any other feature which points to the abdomen, the leucocytes are counted each hour. If the abdominal features are suggestive of perforation, the operation is performed, whatever the leucocytes may show. If, however, there is a rising count, an operation is performed although the local signs may seem insufficient. It is granted that a rising leucocytosis may mean something else than perforation. In one of our cases it was appendicitis. In this clinic practically every case in which either of these features is present is operated upon, under the belief that it is much safer to operate too soon than too late, and that an unnecessary operation affects very slightly the course of the typhoid fever. We have succeeded in this way in saving about 30 per cent. of our cases of perforation. In most cases there is a slight rise of the leucocytes, either an absolute leucocytosis of 10,000 or over, or one relative to the previous counts. Following this rise is sometimes a drop which some suppose is coincident with the spread of the peritonitis. In some malignant cases occurs a fall without any preliminary rise. In those so-called preperforative cases there is a slight leucocytosis due to the local peritonitis. While a great deal of weight is placed upon the leucocyte count no absolute value is allowed it, and it is always interpreted in the light of the physical examination.

Pneumonia.—The coagulation is rapid, as a rule. The count of the *red blood-cells* is normal during the fever, or there is a rise at first, as in Sadtler's case to 7,000,000. After the crisis there is always a drop of about 500,000 cells, sometimes a slight post-febrile anæmia. The hypercythæmia is due to the concentration of the blood, and certainly covers a certain real anæmia caused by the loss of blood to the exudate

and by the destruction of the blood-cells, as shown by the jaundice and the urobilinuria. Cases with these two features show a greater anæmia than others, with a loss of about 2,000,000 cells. The drop which occurs on the day of crisis is partly the disappearance of the hypercythæmia and a drop below normal due to the general peripheral relaxation (Grawitz). In addition to this is a certain grade of true anæmia which sometimes is severe.

Nucleated reds are more common in pneumonia than in other acute fevers. Both normoblasts and megaloblasts occur, which latter have a bad prognostic import only when present in considerable number. At the time of the crisis it is thought the cells crenate more easily than normal, evidence of some chemical irritant in the blood.

In 34 cases in which special attention was paid to the red blood-cells there occurred a drop during the lysis or just after the crisis, generally of about 1,000,000, but in some cases of 2,000,000 cells, which drop usually only restored the count to that level which obtained before the hypercythæmia. The later counts showed small gains and losses in an even number of cases and of about the same degree, but in 9 cases there was a permanent loss of from 900,000 to 1,500,000, and in 4 cases a gain of from 700,000 to 1,900,000.

Pneumonia is the disease in which the *inflammatory leucocytosis* has been best studied. None of our cases showed evidence of an initial hypoleucocytosis, as claimed by Pick. From the first, six to eight hours after the chill, the leucocytes are found increased, and they drop at about the time of the crisis. This leucocytosis is an expression of the resistance of the organism to the infection, and depends but little on the fever and the extent of consolidation. Cabot has divided the cases into three groups: (1) Those with good resistance and a mild infection, in which there may be no leucocytosis; these cases all recover. (2) Those with a severe infection and a good resistance, in which the leucocytosis is high, between 20,000 and 30,000, but in some cases over 100,000, even 115,000 (Löhr). This group includes about 90 per cent. of all cases. (3) Those cases with a poor resistance but a severe infection, in which there is no leucocytosis or even a fall, and which cases are almost always fatal. The best illustrations of this last group are the terminal pneumonias of chronic diseases, pneumonia in the aged and in alcoholics. In fatal cases in which the count does not rise the percentage of polymorphonuclears may rise considerably. In case of a pseudocrisis the statement is made that the leucocytes do not drop, and even rise. The fall in the leucocytes begins just before, just after, or with that of the temperature, and may be preceded by the maximum count. Löper claimed two maxima, the one at onset, the other just before the fall. They fall by lysis rather than by crisis, reaching normal on about the second day. If the temperature falls by lysis, the leucocytes fall as a rule more slowly. In cases in which a slight temperature continues

after the crisis the leucocytes remain elevated until this returns to normal. In fatal cases there is often an ante-mortem rise. In delayed resolution the leucocytes may stay elevated even for weeks and then slowly drop, but in these cases if the temperature has become normal these will as well. For the count to remain elevated should suggest delayed resolution, empyema, or gangrene.

A high count gives no idea of prognosis; it means that the patient is making a vigorous fight, but gives no hint as to which will win, he or the infection.

In our pneumonia cases the leucocytes are counted twice daily. We have compiled the records of some of these cases (158 uncomplicated cases with recovery, 56 uncomplicated cases with death, and 80 cases with various complications), studying them as regards age and termination. In our uncomplicated cases with recovery sex, and the extent of the consolidation have no relation to the degree of the leucocytosis. Age has remarkably little influence; exactly the same percentage of cases below forty years had a leucocyte count below 20,000, as of those older. In all the uncomplicated cases with recovery, 38 per cent. were below 20,000; 7 per cent. above 40,000.

Seventy-seven of these cases terminated by crisis. Of these the cases above 40 years of age had an average leucocytosis somewhat higher than those younger, probably since there were fewer low counts. With 10,000, were no cases; from 10,000 to 15,000, 18 per cent.; these were clinically very mild cases; from 15,000 to 20,000, 25 per cent.; above 40,000, 8 per cent. There was a sharp rise at crisis in 42 per cent. of the cases.

Of 81 cases with lysis the count during the course was below 10,000 in 2 per cent.; from 10,000 to 15,000 in 20 per cent.; from 15,000 to 20,000 in 14 per cent.; above 40,000 in 10 per cent. There was a sharp rise just at lysis in 34 per cent. These rises, which occurred just before the lysis or crisis, were of from 5000 to 10,000 cells, as a rule, but in a few over 20,000, and in one case 30,000. The highest count during the course was 105,500—a young man 25 years old who recovered.

Of the cases with crisis the leucocytes began to drop before the temperature in 15 per cent.; with the temperature in 41 per cent., and after it in 44 per cent. In the lysis cases the drop began before the temperature in 18 per cent.; with, in 43 per cent., and after, in 39 per cent., which are practically the same figures as for those cases with crisis. To reach normal required in the crisis cases from one to twenty days, but the mean time was three days. A well-marked pseudocrisis occurred in 9 cases; of these, 2 were accompanied by a rise of leucocytes, 4 by a fall, and 3 by no change. In cases with a slight fever for some days after the drop the leucocytes remained from 12,000 to 15,000 until the temperature reached normal.

There were 56 fatal cases. The leucocyte counts in these were almost the same for the various decades as in those with recovery. During the course they remained below 10,000 cells in 23 of the cases (in one case they reached even 1700); from 10,000 to 15,000, 23 per cent.; from 15,000 to 20,000, 15 per cent.; and over 40,000 in one case. At the time of death the count was below 10,000 in 17 per cent.; from 10,000 to 15,000 in 25 per cent.; 15,000 to 20,000 in 10 per cent., and above 40,000 in 8 per cent.; all the last cases were under thirty years of age. Toward death in 70 per cent. there was a progressive rise, in 30 per cent. a fall. The absence of leucocytosis is not necessarily fatal. In one case with extreme toxæmia and a count of 8000 the leucocytes slowly rose to 14,000 as the patient recovered.

Daily Variations in the Count.—The two counts were made in the forenoon and afternoon, and separated by an interval of about nine hours. These counts differed by from 1000 to 26,000 cells, as a rule from 4000 to 6000, with a mean of 4000. There was no difference in these variations before and after the crisis. When the temperature was very constant the variation was less marked, the

greatest variation occurring in cases with an irregular temperature, but even then there is no parallelism between fluctuations of temperature and leucocytes, in some cases even a reverse relation.

In cases of delayed resolution, in some the leucocytes reached normal before the temperature; in others both temperature and leucocytes were normal before resolution was complete; again, in others the temperature was normal before the leucocytes.

The cases of terminal pneumonia vary much, our series showing two with counts above 50,000 and two below 3500. Alcoholics had almost no leucocytosis, and yet some recovered. In all cases followed by empyema the leucocytes for the most part showed no change which would indicate when the resolution or the empyema began. In one case throughout the whole disease and to the time of the operation there was no leucocytosis, and in another case the leucocytes did not rise at all until the empyema began. In 2 cases followed by pleurisy with effusion the leucocytes were normal after the crisis (6000 and 8000). In 3 fatal cases ending in abscess of the lung the leucocytes were respectively 46,000, 30,000, and 8500. In 35 cases with various pus infections, endocarditis, pericarditis, meningitis, parotitis, otitis media, phlebitis, thrombosis, tonsillitis, etc., very little could be learned from the leucocytes; that is, there was no notable rise, although the fall may have been delayed. If already low, they did not rise.

Qualitative Changes.—The leucocytosis is a rise of especially the polymorphonuclear neutrophiles; they may be even 90 per cent. of all, but of our cases they were very seldom above 80 per cent., that is, not as high as is general in an inflammatory leucocytosis. The small mononuclears while relatively diminished are often absolutely much increased. During the course the eosinophile cells may disappear, but after the crisis the polymorphonuclear neutrophiles drop to even below 60 per cent., and the eosinophile cells increase, but not to a great degree. Myelocytes appear even in good numbers (almost 12 per cent.), and large basophilic mononuclears. In about three days the percentages are normal. Löper said that if the polymorphonuclear neutrophiles were above 90 per cent. an increase in this percentage meant a bad prognosis; in other fatal cases they may be below 50 per cent.

Glycogen can be demonstrated in the leucocytes nearly always, in amount varying with the temperature and the extent of consolidation, in a marked case the majority of the leucocytes being thus laden.

The *platelets* may even disappear during the continued fever, but after the crisis increase to above normal.

The *fibrin net-work* is increased more than in any other disease. Coagulation is rapid. *Specific gravity* varies as the count and is high. The toxicity of the blood is even double.[86]

The *diagnostic value* of the blood-count is considerable, the presence of a leucocytosis excluding malaria and typhoid fever and suggesting a central pneumonia. It is especially important in the old and in the young, and in cases without localizing symptoms, the leucocytes leading one to search for the physical signs.

In **Intestinal Parasites** a slight leucocytosis is the rule. In 12 of

[86] Albu, Virchow's Arch., vol. cxlix.

our 18 recent cases these cells varied from 11,200 to 34,000. In 4 of the cases with normal counts there was some fever.

Bronchial Asthma.—In bronchial asthma the most interesting find is an eosinophilia of even 53.6 per cent., which is important in diagnosis, and by means of which the oncoming paroxysms may in some cases be predicted.

In 17 cases during the past four years the red count was high, over 5,500,000 in 7 cases; the lowest, 4,900,000. There was a leucocytosis of 10,000 to 15,700 in 6 cases. In but 8 cases were differential counts made, but of these, 6 had above 5 per cent. eosinophiles; maximum, 20 per cent. in a total count of 8600. (Of these six, the absolute numbers of eosinophiles were 728, 712, 535, 856, 702, and 1720.)

Acute Articular Rheumatism.—"The blood is the best index of the severity of this disease" (Osler). Its virus is a rapid and powerful destroyer of the red cells, causing often, but not always, a reduction of from 1,000,000 to 2,000,000 cells. Ewing considers that the anæmia has been exaggerated. The high count which is sometimes seen during the attack some explain as due to the sweats, and call attention to the fact that the anæmia is most evident at the time of convalescence. Hayem, Türk, and others say that the count is lowest at the height of the fever, and that regeneration begins at once with defervescence. It is rare to find nucleated reds. The hæmoglobin suffers worse than the red blood-cells. In no other disease is the fibrin net-work so thick.

Leucocytes are increased, as a rule their count running parallel to the severity and acuteness of the disease. Cabot's average was 16,000. The differential count changes are as in other acute diseases. In subacute or chronic rheumatism there is no leucocytosis.

Of 77 cases of this disease, the red counts were, 2,000,000 to 3,000,000, 3; 3,000,000 to 4,000,000, 15; 4,000,000 to 5,000,000, 45; 5,000,000 and over, 14; mean count, 4,500,000; hence very little anæmia. Of 81 cases, the leucocytes were below 5000 in 1 case, from 5000 to 10,000 in 23, 10,000 to 15,000 in 36, 15,000 to 20,000 in 15, above 20,000 in 6.

Another case, a man fifty-six years of age, was admitted with red cells, 1,720,000; hæmoglobin, 27 per cent.; leucocytes, 12,400, He gave the history of painful, swollen, red joints four weeks before. He recovered rapidly.

Arthritis Deformans.—McCrae, in 33 cases, found the average of hæmoglobin 70.6 per cent.; of reds (29 cases), 4,468,000; the leucocytes, 7600; the differential count normal.

Appendicitis.—In acute appendicitis the rule which our surgeons follow is the same as for all acute abdominal cases. After the first suggestive symptom, or on admission, the leucocytes are counted each hour. With a rising leucocytosis an operation is performed without delay, even though the abdominal signs are very slight; on the other hand with marked abdominal signs the operation is performed, what-

ever the leucocytes may be. If the leucocytes are high but stationary when the patient is first seen, one can wait; but if rising, even slightly, there should be no delay. A normal count means nothing; the case may be mild, very severe, or a well-walled abscess. A high leucocytosis, 20,000 or above, indicates acute appendicitis, probably an appendix full of pus and quite tense. The leucocytes probably fall after it ruptures, at least those cases which have recently ruptured are admitted with low counts or even a subnormal count, even while the process is spreading. In appendicitis 20,000 is a high count, and means pus, gangrene, or peritonitis; above 15,000 means an active process. In fulminating cases there may be death without any reaction on the part of these cells.

In chronic appendicitis with abscess a stationary leucocytosis means a well-walled abscess. In those cases with old abscess the count is seldom above 12,000, and often from 6000 to 7000, but after the operation on such a case the leucocytes will rise at once to about 20,000 or over and then gradually drop. This is perhaps due to exposure of a new area to the infection. If the count remains high it means a pocket is still unopened. While many of our cases with well-walled abscess show a normal leucocyte count, yet these cases also show marked fluctuations for which no explanation is offered. In those cases in which the physical features indicate a rupture of the abscess and a spreading peritonitis, the count may rise or drop, or may fall even to subnormal, or, after a fall, may then again rise, the falling leucocytosis being a worse sign than a high stationary.[87]

In catarrhal appendicitis, and chronic appendicitis without any exudate, there is no leucocytosis.

The red cells show no change except in cases with long standing abscess, in which there may be a secondary anæmia. Da Costa mentions an early slight anæmia in most cases, in some a severe anæmia.

Anæmias of Children.—The study of this subject is of especial importance, since the blood picture is often so different from that in the adult. We have even known a diagnosis of lymphatic leukæmia suggested when the blood was normal.

Children are much more susceptible to all agencies causing anæmia than are adults; the anæmia is more rapid in development, more severe, less easily recovered from. The narrow changes are much more strikingly mirrored in the blood, and when regeneration does begin the picture is quite spectacular, normoblasts, megaloblasts, and myelocytes appearing in such good numbers.

The "*anæmia of growth,*" it is claimed, is explained by the inability of the blood-building organs to keep pace with the growth of the body. At this time wasting diseases, infections, or any agency del-

[87] See Bloodgood, Prog. Med., December, 1901.

eterious to the blood, vascular system, or the hæmatopoietic organs, as poor food, bad hygienic surroundings, influence the child much more than the adult. The development of the heart and of the whole vascular system is also in the closest relation with the blood condition, hence chlorosis and other severe anæmias of youth occur in children with hypoplasia of the cardiovascular system, and this is attributed to a congenital defect. The anæmia of school-children is due to mental strain, lack of exercise, poor appetite, constipation, etc.

A long-standing anæmia in a child is perhaps never perfectly recovered from. Objective evidence of it may disappear and the child seem well, but relatively insignificant agencies will cause it to reappear.

In all such anæmias of the very young a lymphocytosis is usually present and unripe elements appear—normoblasts, myelocytes, and large basophilic non-granular leucocytes—which do not occur in the normal blood. But these qualitative changes in the blood picture have less significance than in the adult, and show more an instability of the blood-regulating mechanism.

The ANÆMIA PSEUDOLEUKÆMICA INFANTUM of v. Jaksch was described as a condition in young children of severe anæmia (even 820,000; most were 1,500,000 to 3,500,000), with low color-index (0.50), a leucocytosis of even 54,660 (in one case, 114,000), with a few myelocytes.

Among the red cells are sometimes many deformed and degenerated ones, and many nucleated reds. The leucocytes are characterized by their great variations in form, size, and staining qualities. The platelets are increased.

The best recent discussion of this condition is that of Cabot,[88] who thinks that the many very different cases thus diagnosed cannot be grouped together. They might be cases of pernicious anæmia, secondary anæmia with leucocytosis (due to lues, rickets, etc.), or Hodgkin's disease, or either of the leukæmias, all of which diseases are apt to be atypical in children.

Bezançon and Labbé emphasize inherited lues as the cause.

Reckzeh, from analogy from experiments on adult and young dogs, considers this form in children a simple anæmia,—that the special features differing from that of an adult are due to the reaction of the young.

Malaria in children causes an especially severe anæmia, with normoblasts and perhaps always megaloblasts, but no marked leucocytosis except perhaps a lymphocytosis.

Congenital lues causes the severest anæmia, with many nucleated

[88] Fifth edition, "Clinical Examination of the Blood," p. 519.

reds and the lymphocytes much increased, the total count being even from 50,000 to 100,000.

Rickets causes a simple chlorotic anæmia, with the leucocytes even 30,000, most of them small mononuclears.

A recent case of anæmia in a fourteen-months-old child is entered on our records simply as "Anæmia with enlarged liver and spleen" (Osler). The red cells on admission were 1,252,000 per cubic millimetre; hæmoglobin, 20 per cent.; leucocytes, 14,700. The child was in the ward one month, and did not improve at all. The leucocytes varied from 13,000 to 26,500, always with the same formula (s. m., 40 to 52 per cent.; l. m. and tr., 5 to 18 per cent.; pmn. n., 38 to 62 per cent.; eos., 0 to 0.9 per cent.; neutroph. myeloc., 0.9 to 3 per cent.; Mastzellen, 0 to 0.2 per cent.; nucleated reds, 24 to 250 per 1000 leucocytes, chiefly normoblasts, some intermediates, and megaloblasts and microblasts).

Such a case resembles the French "splenomégalie chronique avec anémie et myélemie."

SUMMER DIARRHŒAS OF CHILDREN.[89]—The ordinary summer diarrhœas are usually accompanied by a leucocytosis, but of so wide a range that it has no diagnostic value. In the simple dyspepsias the differential count of leucocytes is normal (total, 13,500 to 36,000; s. m., 39; l. m. and tr., 21.2; pmn. n., 37.8; eos., 2), but in the more severe cases there is an increase in the polymorphonuclear neutrophiles (56 to 63 per cent.), a decrease of the mononuclear cells (33 to 7 per cent.), the blood thus presenting the picture of an adult blood. In an acute intestinal toxæmia and the severe forms of enterocolitis occurs a true leucocytosis.

A leucocytosis with a wasting disease in a child usually indicates an inflammatory intestinal complication.

Myelocytes are few, eosinophiles sometimes disappear. The leucocytosis is no indication of the severity of the disease, but the percentage of the various cells is.

In some cases of severe diarrhœa the red count may rise even to 10,000,000 cells.

CHRONIC DISEASES

Chronic Nervous Diseases.—These present nothing at all characteristic in the blood; in these diseases its condition depends more on the general nutritional condition of the patient.

Some cases of CHOREA are very anæmic, but the chorea is probably not the primary condition. In 23 cases of this clinic there were but three with a mild secondary anæmia, the lowest 3,400,000 reds, and these were also heart cases.

There is sometimes an eosinophilia of from 7 to 10 per cent. (Thélème).

In GENERAL PARESIS Capps and Jenks[90] found in some cases just

[89] Knox and Warfield, Johns Hopkins Hospital Bull., July, 1902.
[90] Am. Jour. of Insanity, January, 1900; Diefendorf, loc. cit., 1903, vol. cxxvi.

before a paretic seizure an absolute leucocytosis, with an increase especially of the large mononuclears. In this condition is also some anæmia which progresses with the disease, except during the seizure, when is seen a temporary rise of reds.

In MANIACAL DEPRESSIVE INSANITY Fisher[91] found often an anæmia, almost always a leucocytosis during the periods of excitement, but no pathognomonic blood-changes.

In ACROMEGALY there is an increase in reds with eosinophilia and lymphocytosis (Ducati).

Diabetes Mellitus.—The symptoms of this disease are essentially those on the part of the blood, and the urinary ones are only secondary, the glycosuria being the result of the hyperglycæmia, the sugar in the blood reaching even 0.57 per cent. instead of, as normally, 0.1 to 0.2 per cent.

Concerning the red blood-cells the reports are various. The hyperglycæmia causes an hydræmia, and hence dilution of the blood; diuresis, on the other hand, concentrates the blood, hence the actual count found varies considerably. Later in the disease, however, as the cachexia develops there is an anæmia, and yet this anæmia may be well masked in a concentrated blood. The leucocytes are normal; the patients show a remarkable digestive leucocytosis.

In 45 cases, the red cells were below 4,000,000 in 3 cases; the lowest was 2,000,000; from 4,000,000 to 5,000,000, 13 cases; 5,000,000 to 6,000,000, 19 cases; over 6,000,000, 4 cases. Three other cases were at times over 6,000,000.

Of 40 cases, the leucocytes in 25 varied from 5000 to 10,000; from 10,000 to 20,000 in 7 cases; over 20,000 in 7; the highest, 44,000. The explanation of the leucocytosis was pneumonia, septicæmia, furunculosis, gangrene; and in a case of coma they varied from 30,000 to 41,000.

LIPÆMIA is common especially in severe cases. In this condition the serum is milky from the increased fat in the blood, this being present in dust-like particles, which are sometimes coarse; the fat is increased greatly above the normal amount. Bönniger found 0.75 to 0.85 per cent.; normal, 1 to 3.25 p.m.; others in diabetes from 30 to 117 p. mille; yet lipæmia may exist with but 5.5 p.m., and we cannot claim an exact relation between the visible and total fat. Lipæmia is physiological in sucklings, in very obese persons, in some pregnant women, and in adults after a heavy fat meal; but the best examples are severe cases of diabetes mellitus with considerable sugar excretion, even after a fast of twenty-four hours. It may exist for several weeks before death.

The cause is perhaps disturbed oxidation of the fat present, probably the fat of the diet, while others claim the sugar is directly transformed to fat. Lipæmia occurs also in cases of chronic alcoholism,

[91] Am. Jour. Insan., April, 1903.

pneumonia, in progressive tuberculosis, in fracture of long bones, in contusions of subcutaneous fat, and, it is said, in some cases of liver and kidney disease, malaria, cholera, and phosphorus and carbon monoxide poisoning. A recent article on lipæmia is by Futcher,[92] and by Fraser,[93] in whose case there was 16.44 per cent. of fat in the blood and 18.94 per cent. in the pleural exudate. The record case is Fischer's, with 18.129 per cent. in the blood.

Bremer's blood-test has proved valuable in certain cases. A thick smear of blood is made on a slide, and a similar one for a control of normal blood. These are subjected to exactly the same treatment. They are first heated to 135° C., then allowed to cool slowly, and are stained with 1 per cent. Congo red, aqueous solution, for two minutes. Relative to the normal blood the diabetic blood will take a yellow rather than a red tint. This test is positive also when the urine is sugar-free, and is said to be given even before sugar has appeared. Schneider found it present in two normal men who were great meat eaters, and ascribes it to the reaction of the blood. Strauss confirms this opinion, finding it best in cases of acidosis. It is claimed to be sometimes present in cases of leukæmia, Hodgkin's disease, and Graves's disease, and yet, granting this, it is of importance in diagnosis.

In this clinic a man was admitted during coma; no urine could be obtained by catheterization; the diagnosis of diabetic coma was made from this test alone, and confirmed later by autopsy.

Williamson's Test.—Twenty cmm. of blood in a test-tube are mixed with 1 cc. of aqueous methylene blue (1 to 6000); 40 cmm. of 60 per cent. KOH and 40 cmm. of water are added. This mixture is allowed to stand for three or four minutes in boiling water. If the blood be diabetic it takes a yellow color, which is said not to be due to the sugar in the blood.

Quantitative Determination of Fat in the Blood.—The method chosen by Bönniger, in Salkowski's laboratory,[94] is as follows: From 5 to 30 gm. of blood are mixed with 10 to 20 volumes of 96 per cent. alcohol, the precipitate ground fine, then allowed to stand one or two days. It is then filtered, the precipitate again extracted several times with alcohol in the same way, then with 5 to 10 volumes of ether twice, digesting one day each time. All these extracts are then combined, evaporated, repeatedly taken up in absolute alcohol, and this evaporated, then filtered, dried, and weighed.

Clinically, sufficiently accurate results are obtained by extracting twice with alcohol alone; this will give 96 per cent. of the total fat.

Malignant Disease.—Malignant disease is one of the most important causes of anæmia. This is because of the frequent hemorrhages, the mechanical effects of the cancer as is seen in those of the gastro-intestinal tract, and, most important, the cancer toxine which may cause an extreme anæmia even though the tumor be small and cause no local symptoms; be, indeed, latent. The result of the growth is an anæmia

[92] Jour. Am. Med. Assoc., October 21, 1899.
[93] Scot. Med. and Surg. Jour., 1903, p. 200.
[94] Zeits. f. klin. Med., 1901, vol. xlii.

parallel to the progressive cachexia. And yet it is remarkable how long the blood will present an almost normal picture before the slight anæmia begins, and then how rapidly the anæmia and cachexia will develop. Grawitz claims that the cancer produces a plasmotropic poison; that is, one which may affect the blood, or the blood plus the body tissues, or the tissues without the blood, producing in some cases merely degenerations of the red blood-cells; in other cases an anæmia parallel to the cachexia; in still other cases a marasmus of the highest grade yet with the blood only slightly affected.

Cancers vary much. Some, for instance those of the skin or lip, cause no anæmia, while a fulminating cancer, as of the stomach, "may give a perfect picture of primary pernicious anæmia, or, indeed, of leukæmia." In general it is stated that the more malignant the tumor the greater the blood changes, the more extensive the cancer, that is, the more its metastases, the greater its influence upon the blood. But this is not entirely true: our cases with rapidly developing metastases, with large nodules, are those with a slight chlorotic anæmia; those which simulate pernicious anæmia are more often those with few objective signs of cancer, an insignificant looking little nodule.

The common picture is the so-called "pseudo-chlorosis carcinomatosa;" others show the picture of the severest pernicious anæmia, the cases being thus diagnosed, and the diagnosis corrected at the autopsy by a small cancer nodule, often of the stomach, which before that was unsuspected. In still other cases no anæmia results. Cabot says in one-half the cases there is a chlorotic anæmia; in about one-fourth, none; while one-fourth show a reduction in both count and hæmoglobin.

The severest anæmia occurs in those cases with frequent hemorrhage, as, for instance, of the stomach and of the uterus. Anæmia from cancer of the digestive tract is sometimes great because of the resulting malnutrition. v. Limbeck says that blood normal qualitatively is common and perhaps the rule, even in advanced cases; that in cases with desiccated tissues there may be even a rise in the count; and in advanced cachectic cases without hemorrhage there is seldom diminution in the red count, and then it is not extreme.

The anæmia when it does occur is of the secondary type as a rule, and severer than that due to any other chronic disease. The chief changes are at first in the size, shape, weight, and degeneration of the red blood-cells; later, as the cachexia develops, the anæmia is often as low as 2,500,000 cells, or even as low as in pernicious anæmia, 1,000,000 cells. An exception to this is in cancer of the œsophagus, where, indeed, the blood may be concentrated.

Hæmoglobin is always and is first reduced. In some cases it may be of a normal percentage, but in these the count is above normal; the average in long-standing cases is about 68.5 per cent., in worse cases 57.5 per cent. It is, therefore, rarely as low as in chlorosis. This low hæmoglobin is an important diagnostic point between malignant and non-malignant tumors. Cabot's average was 58 per cent., with an index of 0.65. The hæmoglobin is lower in cases of visceral than peripheral cancer, and the index is low even in severe cases; yet Bezançon and Labbé mention two cases in which the cells were increased in size and with an index over 1. After operation regeneration begins at least one week later than would be expected, and it is said never quite to regain the percentage it was before operation, even though the patient gains in weight (Bierfreund).

There is always diminution in the average size of a few or most of the cells. The giant cells of pernicious anæmia are rarely seen here except late, while microcytes are common (Grawitz). The basophile granulation is very common. The deformities in size and shape and all degenerations may be absent or they may be even more marked than in tuberculosis, in which case they are of diagnostic value; they are a less prominent feature, however, than of pernicious anæmia. Nucleated reds are present as a rule and always in the severe cases. They are more numerous than in secondary anæmias due to any other cause, and are found even when the anæmia is slight. Their presence has some diagnostic value in a differential diagnosis between cancer and ulcer of the stomach. They are normoblasts, as a rule, although in those cases which simulate pernicious anæmia a few megaloblasts may be present. In cases involving bone-marrow their number may be enormous, even 90,000 per cubic centimetre (Türk).

The blood is hydræmic, with reduced albumin. Metabolism experiments indicate an abnormal destruction of tissue proteid, the evidence of a circulating toxine. Grawitz suspects that in some cases the anæmia is only apparent, since injection of carcinoma extract shows that the tissue lymph flows into the blood, thus diluting it; others doubt. Much of the anæmia is due to repeated hemorrhages, which are common enough, yet there is all the evidence necessary of a hæmolytic toxine.

Leucocytes.—In general, in about 60 per cent. of all cases, there is a moderate leucocytosis, an important point in the differentiation of benign and malignant tumors, and this may be the first sign of cachexia. Of some the count is normal; some present the appearance of leukæmia. This leucocytosis depends upon the hemorrhages and the position of the cancer. In œsophageal stricture the starvation will sometimes cause a leucopenia, and the cells present will be chiefly lymphocytes; in cancer of the uterus and stomach so commonly ac-

companied by hemorrhage, a post-hemorrhagic leucocytosis is common; in those of the thyroid, pancreas, and kidney the count is especially high. It also depends upon the size of the cancer, including of course the metastases; the larger and the faster the tumor grows the more the leucocytosis; but there are great variations. Grawitz, from his injection experiments with cancer extract, considers that the increase is due to the entrance of the tissue lymph into the blood-stream, carrying with it a great many leucocytes, the same which occurs in a post-hemorrhagic leucocytosis, and hence considers that the leucocytosis is coincident with the softening of a tumor mass. After operation the leucocytes drop, and their subsequent rise may indicate a recurrence even before it can be found physically.

Differential Count.—As a rule, a leucocytosis means an increase of the polymorphonuclear neutrophiles, but in some cases these are low, even 43.7 per cent., and the lymphocytes are increased. In other cases is a leucopenia of even 3000, but with the polymorphonuclear neutrophiles even 88.7 per cent. The eosinophiles are not usually as much diminished as in other leucocytoses, but, on the other hand, they may not be increased even when bone metastases are present. Myelocytes are more commonly present in cancer than in any other condition excepting leukæmia and pernicious anæmia.

The specific gravity of the blood is low. The plasma is rich in sugar, even as rich as in diabetes. Its alkalinity is decreased even to one-third. The coagulation is normal or retarded unless sloughing or inflammation be present, in which case it may be rapid. The fibrin net-work is usually normal.

It is said that the effect of cancer is seen in degenerative changes of the leucocytes before the quantitative ones begin.

In cancer of the breast a slight leucocytosis (11,000) is common. In cancer of bone are found very many nucleated reds, normoblasts and megaloblasts, and leucocytosis with a high percentage of mononuclears, and some myelocytes.

Cancer of the Stomach.—It is particularly the cancers of the stomach which cause an anæmia simulating the primary pernicious. The count may be very low,—*e.g.*, a case of Menetrier and Aubertin, 1,333,000; Grawitz's case, with 500,000 reds; yet these cases are rare, Cabot in 129 cases finding 27 with counts above 5,000,000, 26 below 3,000,000, the average on first visit being 4,018,000. Of the 134 cases of this clinic, including those reported by Osler and McCrae, 33 were above 5,000,000, 16 below 3,000,000, mean about 4,000,000. The color-index is always considerably below unity unless the count be very low. Nucleated reds were rather rare. The count sometimes drops progressively till death (in one case to 1,786,000). The high counts are sometimes attributable to the vomiting. The differential

diagnosis between cancer of the stomach and pernicious anæmia is one of well-recognized difficulty, and in many cases can be settled only at autopsy. In general it may be said that in pernicious anæmia there is a lower count; that if the red blood-cells are below 1,500,000 it is against cancer; yet this rule is a broken reed, for it fails in both directions. Cases most like pernicious anæmia have small insignificant cancer nodules, and without autopsy would pass as primary anæmia. After autopsy one can by retrospect see minor points which should have led him to suspect cancer, but only then. In cancer the index is generally below one; there are fewer nucleated reds and those that are present are normoblasts as a rule; and leucocytosis is more common. In cancer the red blood-count is always higher than the cachexia would lead one to suppose, in pernicious anæmia the reverse. The blood examination Henry thinks of greater value in this differential diagnosis than the gastric analysis. The leucocytes in this disease vary so greatly and so without apparent explanation that little value can be placed on this count, except that there is a leucocytosis in over one-third of the cases, and in those without it the digestive leucocytosis is often absent (in 82 per cent. of 144 cases, yet this is of little real practical value, although it is one point to consider—Da Costa). Low counts, below 4000, are not rare; our highest was 52,800.

It is said that the rapidity of growth controls the count, and yet our lowest counts included those certainly with metastases in liver, pancreas, or peritoneum (1600, 5400, 5000, 5600), and in 15 cases of cancer of the liver or abdominal organs generally the leucocytes were above 10,000 in but 7. In one case, 105,000 (t° 103° F.); in another, 24,500 (t° 99° F.); just before death in a third, 61,400. The high counts were nearly all in those with the slight fever so often present.

Cabot reports a case with perforation into the peritoneum followed by quickly fatal peritonitis and a count of 105,600. We suspected this condition in a case the count of which rose to 120,000, an almost pure leucocytosis, but were unable to get an autopsy.

The percentage of large mononuclears has been found rather high (1 to 10 per cent.); in one case before death even 33 per cent. of a total count of 6300 (Kurpjeveit).

In carcinoma of the œsophagus the blood is concentrated, giving a high content of dried substances; in v. Noorden's cases, 26.5 and 27.3 per cent., yet even in those cases there may be an oligæmia. If the cancer extends to the larynx, causing dyspnœa, the count may be high from the cyanosis.

Of our 6 recent cases, in one case the red count was 5,960,000, the lowest 4,184,000, and in another the first blood examination gave 4,696,000, 85 per cent. hæmoglobin, 6000 leucocytes; a later count was 6,476,000, 104, and 19,000 respectively. Five of the cases showed a leucocytosis, the highest 30,250. In 15 cases of cancer of the liver or general of the abdominal organs, cases in which all conditions were favorable for rapid and extensive growth, it is interesting that in but two was the red count below 4,000,000. In 27 cases of cancer of the bile-ducts

the lowest red count was 3,700,000, and in five the leucocytes above 10,000; in one case, 44,150 (t° 100° F.).

In 4 cases of CANCER OF THE RECTUM the reds were scarcely reduced (lowest, 3,732,000, the rest about normal). There was a leucocytosis in two, of 13,100 and 19,750 (t° 100° F.).

In 10 cases of CANCER OF THE INTESTINE 3 showed marked anæmia; in one, reds 1,600,000; hæmoglobin, 40 per cent.; leucocytes, 2500; situation, the ileum: the other had 1,780,000 reds, 28 per cent. hæmoglobin, and 10,000 leucocytes; this patient was a nephritic as well: the third, reds, 1,609,000; hæmoglobin, 40; leucocytes (one week before), 7500; situation, the sigmoid flexure. The other red counts were from 4,000,000 to 4,500,000, four cases; the highest, 5,348,000; but 2 of the 9 showed a leucocytosis.

Our other cases of carcinomata showed no striking features, except one of the TESTICLE, with 2,832,000 red cells and 9600 leucocytes. Cancers of the kidney show usually high leucocyte counts, even 54,000. We had no such case. Also of the thyroid (71,000), and some of bone (52,700).

In **sarcoma** there is in general the same condition as in carcinoma, but some think that the effect on the blood is worse. We could not believe this from the study of our cases. This may be true particularly if the bone-marrow or the lymph-glands are especially involved; then a severe anæmia and high leucocytosis are the rule. In a case of osteosarcoma the red blood-cells were 663,400 (Hayem); in another case, 1,118,000; hæmoglobin, 28 per cent.; leucocytes, 68,-200: in still another case, 2,240,000; hæmoglobin, 48 per cent.; and leucocytes, 54,000 (the last two of v. Limbeck). Yet other cases have counts even above 6,000,000, while still others present the appearance of a primary pernicious anæmia. The nucleated reds are said to be less numerous than in carcinoma. The hæmoglobin is said to be more reduced than in other cancers, the average being about 50 per cent., with 30 per cent. not rarely, while cases even below 10 per cent. are reported. The leucocytes in cases of osteosarcoma average about 17,000. They are more constantly increased and to a greater degree than in cancer, the cases resembling leukæmia. Among the qualitative changes an increase in the polymorphonuclear neutrophiles is less common than in cancer, but it may be present when there is no leucocytosis. This is said to have diagnostic value as against cancer. In some cases there is a large percentage of eosinophile cells, even 50 per cent., with little evidence that it is due to bone metastases. Myelocytes are sometimes increased. The old question whether all these cells are leucocytes or free sarcoma cells recurs often, for the increase often seems due to the small mononuclears.

Lues.—Lues, according to v. Limbeck, is the best illustration of

the dictum that no blood picture can be considered characteristic for the anæmia due to any one disease. In this case the blood picture may be the most varied, simulating anything from chlorosis to pernicious anæmia. The anæmia is particularly striking in the case of women. At first of the chlorotic type, it may at the end simulate a pernicious anæmia even of the severest grade, with a count of 428,000 cells; some cases of acquired lues have a practically normal blood, but this is unusual.

It is important to separate the effect of the disease as such from that of the overtreatment with mercury. In an untreated case this chlorotic condition develops to a degree which depends on the duration and on the severity of the infection, then will gradually improve, with the improvement in general condition spontaneously, or more rapidly if the case is then treated.

During the *primary stage* a severe chlorotic anæmia is the rule, and any one following large European skin clinics is struck by the importance that is placed upon this anæmia, particularly in the case of women, in the diagnosis of a primary sore. Some say that the count will remain normal while the hæmoglobin diminishes considerably. During the transition from the primary to the secondary stage, one of the first signs of the dissemination of the disease throughout the whole body is the appearance of the rash and a further diminution of hæmoglobin. Yet the count remains nearly normal, while in a few days there may be a loss of hæmoglobin of 25 to 30 per cent. If untreated, the percentage may soon be as low as 25 per cent., and red blood-cells also drop, even 230,000 cells per day. The severity of the anæmia depends on the condition of the patient, age, treatment, etc. In well-treated cases a rapid regeneration follows.

Leucocytes.—During the tertiary stage with severe anæmia there is often a leucocytosis with a high lymphocytosis. Myelocytes are present in a severe case. This leucocytosis is an aid in excluding pernicious anæmia.

In an adult a high lymphocytosis and an eosinophilia suggest lues. In the case of a child it could also be rickets. A low hæmoglobin with high percentage of small mononuclears indicates a severe case.

In the primary stage the leucocytes are normal, or there is a slight leucocytosis with an increased percentage of lymphocytes. If mercury be given the percentage of the polymorphonuclear neutrophiles rises, the reverse of the action of mercury in a normal case.

During the secondaries the leucocytes vary from 12,000 to 16,000, the lymphocytes and eosinophiles are increased, the latter especially with the papular syphilide.

In 19 cases of secondary lues the red cells were diminished but slightly (minimum, 4,200,000; above 5,000,000 in 6), the hæmoglobin was evidently more affected (40 to 90 per cent.; mean, 75 per cent.; color-index, 0.5 to 0.9; mean, 0.7), the leucocytes, as a rule, were normal (in 11 cases below 10,000, in 3 between 10,000 and 12,000), except in cases of high fever (luetic fever of secondary stage), of which there were 5 cases, in four with the leucocytes between 12,000 and 24,000, and dropping with the temperature.

There was a slight rise of leucocytes during the primary stage (average 9000). During the secondaries the count depended on the skin lesion and the fever, varying from 9000 to 24,000, or even 50,000; average, 12,000 to 15,000. During the tertiary stage the count varied much, sometimes a slight rise, sometimes leucopenia. In hereditary lues the count has been found high,—12,000 to 24,000.

In tertiary and hereditary lues the reds are more seriously affected both in number, size, weight, and color. Megaloblasts are common. The picture may resemble that of the primary pernicious type, especially old cases with much scarring of organs and sclerotic bone-marrow. Some cases reported in children are probably of anæmia pseudoleukæmia infantum, and yet here the megaloblasts do not predominate. Miller reported a case with 720,000 reds, 18 per cent. hæmoglobin, with normoblasts, megaloblasts, even gigantoblasts, microcytes, and poikilocytes. In the hereditary lues of infancy the anæmia may be fatal. The average leucocyte count of 25 cases was 7050. Large nucleated reds containing little hæmoglobin are important (Cima).

Following mercurial treatment the cells rise even 100,000 cells a day, the rise sometimes ending in even a slight hypercythæmia. But the rise is often preceded by a drop (since those red cells injured by the disease are first destroyed by the mercury?), hence sometimes a hæmoglobinuria and a drop in the count immediately after the inunction, followed by rapid regeneration and improvement in the general condition. If the treatment is carried too far it may itself be the cause of an anæmia. The length of treatment is therefore set at twenty-four days by some (Gaillard), but the individual variations are considerable. Conried advised from twenty-five to thirty-five inunctions; others say to give these *ad libitum*. In general the count rises for fourteen days, and if the mercury is continued begins to fall in twenty-four days.

Of 23 of our cases, 7 of which were of cerebral lues, the red cells were above 5,000,000 in 9 cases, between 4,000,000 and 5,000,000 in 10, and the lowest count 2,870,000. The color-index varied from 0.4 to 0.9; mean, 0.67. The leucocytes were below 10,000 in 20 of 29 cases. They varied from 10,000 to 18,500 in the other 9 cases; 6 were cases of high luetic fever; one was the malignant type (16,800, no fever); the case with 18,500 had large gummata. In one cerebral case the leucocytes were 3000, in another 2100.

Justus's Test.—A large inunction or injection of mercury given

a case of lues before the rash, yet at a time when the general enlargement of the lymph-glands shows that the toxine is now disseminated throughout the whole body, is followed by an immediate drop in the hæmoglobin of from 10 to 20 per cent., and then a rise to normal or even above normal, in from one to several days, with improvement of all the symptoms. This drop, which is both rapid and considerable, is specific for a case of florid lues (but Cabot found it in a case of chlorosis). It may be obtained in any form of lues, late primary, secondary, tertiary, or hereditary, provided the disease be at that time florid, but not when or just before the symptoms begin to recede, as with cicatrization, desquamation, etc. It may again be obtained in case of relapse and until it has passed its climax. It is not present during the primary stage, so long as the infection is limited to the chancre and its neighboring glands, but only after the toxine has become generally spread, as shown by the swelling of distant glands. It cannot be used early to differentiate a hard and a soft chancre. This will explain the dissatisfaction many have with the Justus test.

As explanation it is thought that the mercury kills off the damaged red blood-cells which are soon replaced by new ones.

This test which promised so much has been repeatedly tried by various men. Many fail to get it in cases which turn out to be lues, yet in nearly all of these cases the test may have been applied too soon. Others have found the test in other diseases; for instance, Brown and Dale, Jones, Da Costa, Cabot, and Huger. Justus has lately reiterated his claim for its specificity,[95] and certainly answers well his critics. While the test may not be pathognomonic, it is still valuable.

Renal Disease.—The kidneys play an important part in the control of the composition of the blood, hence in nephritis the plasma changes are early and most important: a loss of albumin, lowered specific gravity, and in general all the signs of a chronic secondary anæmia. This may in part be due to the actual loss of albumin in the urine, the "serous hemorrhage" of some writers, but in acute cases, perhaps chronic, there is evidence of the presence of a toxine.

In the ACUTE HEMORRHAGIC NEPHRITIS especially the count may be very low, even 1,000,000, but usually the anæmia is moderate, and of this much is only apparent.

In 12 recent cases of ACUTE NEPHRITIS there were but two low counts, 2,600,000 and 2,900,000; a leucocytosis in five, 11,400 to 18,900. In 50 cases of Cabot the lowest red count was 3,568,000, but the leucocytes were above normal in 31 of the cases; highest count, 50,000. Cabot thinks the leucocytosis due to loss of blood by the kidneys or to uræmia; but nephritis is an acute febrile, perhaps infectious disease, and we think the leucocytosis may thus be expected.

[95] Deutsches Arch. f. klin. Med., 1903, vol. lxxv. p. 1.

In CHRONIC NEPHRITIS so many factors come into play that the blood picture is not clear. Yet the influence of nearly all these factors is to cause anæmia. Among them is the condition of the heart; the œdema and hydræmia; the wretched condition of the gastro-intestinal tract, vomiting, diarrhœa, poor appetite, and the influence of the purges. The result is often a lowered count, a still more lowered hæmoglobin, and an hydræmic plasma.

In 103 cases of chronic nephritis the red cells were 1,700,000 in one case, between 2,000,000 and 3,000,000 in 13 cases, between 3,000,000 and 4,000,000 in 25, over 5,000,000 in 19; mean, 4,500,000.

The hæmoglobin in 99 cases was between 20 and 30 per cent. in 3 cases, from 30 to 50 in 29, above 80 per cent. in 17; mean, 62 per cent.; hence the mean color-index, 0.7, which is almost normal.

The leucocytes in 80 cases without uræmia were below 5000 in 4 cases, 5000 to 10,000 in 43, above 10,000 in 33, and of these the highest were between 20,000 and 30,000.

In 33 cases with uræmia the highest was 25,900, above 10,000 in 15 cases, below 5000 in 2; mean, about 9000. It is seen that in our cases those with uræmia did not differ much from those without.

A most interesting group is of the cases somewhat resembling pernicious anæmia, and with only nephritis to explain the condition.

A case from this clinic [96] is a good illustration of this, or of the simultaneous occurrence of the two diseases. The patient was a man 39 years old; red cells, 1,400,000; hæmoglobin, 27 per cent.; leucocytes, 7000 (pmn. n., 88 per cent.; s. m., 8 per cent.; l. m., 2 per cent.; eosinophiles, 2 per cent.). There was no poikilocytosis, and but one nucleated red found. The urine was of low specific gravity, with much albumin and many casts.

Cabot reports such a case with 1,468,000 reds; hæmoglobin, 23 per cent.; leucocytes, 3800 (pmn. n., 70 per cent.; s. m., 23 per cent.; l. m., 4.4 per cent.; eosinophiles, 2.6 per cent.; megaloblasts, normoblasts, and poikilocytes).

In the case of Labbé the red blood-count was 500,000; hæmoglobin, 2 gms.; the cells pale, irregular in form and size; nucleated reds rather small; mononuclears, 59 per cent. Recovery was rapid. He suggests that the anæmia was for the most part that of dilution. In another case the red blood-cells were 418,500 and the color-index over 1; and in a third the count was 1,000,000. At autopsy in such cases nephritis is the only lesion found. There were practically no signs of blood destruction, nor of regeneration, nor of megaloblastic degeneration of the marrow. It is a question how much of the low count the hydræmia will explain, but there is certainly some relation between the anæmia and the œdema, and the hydræmia which accompanies the anæmia.

We mention two other cases with arteriosclerosis and chronic nephritis; the one, a woman, fifty-four years of age, reds, 2,800,000; hæmoglobin, 50 per cent.; leucocytes, 6000; no fever; the other, a man thirty-two years old, reds, 1,772,000; hæmoglobin, 22 per cent.; leucocytes, 50,000 (of which 91 per cent. were pmn. n.). The leucocytes later rose to 116,000; he left the hospital unimproved.

In interstitial nephritis the count is normal at first, and sometimes to the end. The condition of the heart is important. During the acute exacerbations, however, a slight lowering of the count is common, perhaps due to the hydræmia.

[96] McCrae, Johns Hopkins Hosp. Bull., October, 1902, p. 245.

In BILATERAL CYSTIC KIDNEY there was anæmia in both of 2 cases, 4,200,000 and 2,800,000; a leucocytosis of 13,500 and 36,000.

Diseases of the Liver. CATARRHAL JAUNDICE.—"Occasionally there is a slight leucocytosis at the onset, otherwise normal blood, with some degenerative changes in severe cases" (Cabot). An increased resistance, rigidity, and size is claimed for the red cells.

Of 27 of our cases, the red count was normal or even above normal in 16; the lowest, 3,000,000; the mean, 5,000,000 in the male patients. An interesting feature was the rise in the count of 300,000 to 750,000 cells while in the hospital. Of the 27 cases, in 20 the leucocyte count was 10,000 or below; in 3, from 10,200 to 10,500; and in 4 from 14,200 to 19,500; these cases all with slight temperature. The cells fell rapidly to normal after admission. A leucopenia follows in some cases (Bezançon and Labbé).

The plasma of the centrifugalized blood is bile-stained. Coagulation time slow.

TOXIC JAUNDICE.—Of this we had three fatal cases. Of one, the red cells were 3,570,000; hæmoglobin, 65 per cent.; leucocytes, 11,400: the second, 5,280,000, 75 per cent., 7000: and the third, 5,400,000, 65 per cent., and 12,500 respectively.

GALL-STONES.—A mild leucocytosis during the attack of colic is very common, a high one rare. During the colic the count in our cases (36 in number) took a sudden jump to about 15,000, but in cases of stone in the common duct with the chills and fever, it was higher, even 24,700. The red cells varied from 2,800,000 to 6,400,000; mean, 4,300,000. In a case with hemorrhage they fell to 1,880,000; hæmoglobin, 23 per cent.; leucocytes, 17,500. The coagulation time should be tested before any proposed operation, and if found increased, a long course of calcium chloride treatment until it is normal is advised.

CHOLECYSTITIS.—The leucocytes are invariably high, from 20,000 to 27,000 (Bloodgood). In one of our cases, 46,500. As the case becomes chronic the count falls nearly or quite to normal.

CHOLANGITIS.—Of 5 cases the leucocytes were 16,000, 33,160 (fatal), 15,600 (t.° 103.5°), 9000 (t.° 103°), and 6,400 (t.° 106°—fatal).

ABSCESS OF LIVER.—During the acute process the leucocytes may be high, but later are lower, or normal when the temperature is normal. Futcher[97] found the average in 15 cases to be 18,350, the maximum 53,000. The reds were 2,600,000 to 5,600,000, mean, 4,200,000; mean of hæmoglobin, 60 per cent.

CIRRHOSIS OF THE LIVER (ATROPHIC).—Early there is no change in the red cells, later an anæmia. Da Costa's average, 3,404,000; Cabot's, 3,580,000; and one case, 1,300,000. The leucocytes are normal or low. In our 32 cases the red cells varied from 3,100,000 to

[97] Jour. Am. Med. Assoc., August 22, 1903.

5,900,000, mean, 4,500,000; hæmoglobin mean, 68 per cent. Leucocytes in 30 per cent. of cases, over 10,000; the highest, 16,000.

Hypertrophic Cirrhosis (of Hanot).—Hayem reported a case with extreme anæmia. We have had five cases; in 2 the count was high, 7,800,000 and 8,500,000; and in one as low as Hayem's case, 1,504,000; hæmoglobin, 28; leucocytes, 6100 (the count rose later). In two there was leucocytosis (11,000 and 12,800).

Acute Yellow Atrophy.—The cases reported have had normal red blood-counts and moderate leucocytosis. In a recent case of this clinic, a boy 14 years old, the reds were 4,800,000 and leucocytes 12,700.

Leprosy (v. Limbeck).—Early no change is noted, but after a few years develops usually a pseudochlorosis with a normal count. After general malnutrition begins the anæmia becomes more marked, and yet is rarely very severe (in one case, however, 2,290,000 red blood-cells, 55 per cent. of hæmoglobin). Leucocytosis has not been found, these cells varying from 4000 to 8000 per cmm.

Heart Disease.—While compensation is good the blood is normal, but with acute loss of compensation and a low blood-pressure the blood is hydræmic, hence the count is low. With, however, the chronic stasis which follows, and the cyanosis, the blood-count rises and may conceal an anæmia. The worst anæmia is seen in aortic valvular insufficiency, as in a case with reds, 3,400,000; hæmoglobin, 30; leucocytes, 8000; and if the blood condition improves the heart may regain its compensation. In congenital heart disease with extreme cyanosis the condition of the blood is particularly interesting, as it is in these cases that we get not rarely a polycythæmia with red cells between eight and nine millions.

During the loss of compensation in 29 males with *pure mitral disease* the count varied from 3,000,000 to 7,500,000, and the mean 6,200,000. In 46 women the mean was 4,700,000, but the extremes, 3,500,000 and 8,000,000. There were interesting jumps of from 1,000,000 to 2,000,000 cells while under treatment.

In 37 cases of *pure aortic disease* the mean was 5,200,000; these cases showed a lower blood-count, as a rule, on each admission.

In 29 cases of *arteriosclerosis* (no important cardiac lesions) the mean was also 5,200,000. In 34 cases of *aneurism of the thoracic aorta* the mean was 5,500,000; in five men with aneurism of the abdominal aorta, 4,500,000.

Addison's Disease.—A hypocythæmia is the rule, the cells varying from 2,000,000 to 3,000,000; in one case 1,120,000; in other cases, however, the reverse is true, and the count may be even above 7,000,000. Some consider that the anæmia is due to a complication, and not to the disease itself. Others, that there is always an oligæmia,

but that this is covered by the concentration of the blood, and cite a case with true oligæmia and a count of 4,774,000 cells.

Myxœdema.—In myxœdema the count may be increased, diminished or normal. Many find an anæmia which improves with treatment. Some have found an increased diameter of the red blood-cells, which decreases under treatment, also many nucleated reds; that is, an infantile condition of the blood. The platelets were in a recent case much increased.

Rickets.—Anæmia is the rule, generally of a mild grade, but sometimes intense, rapid, and even pernicious.

Scurvy.—The count varies generally from about 3,000,000 to 4,000,000 cells. If the case is accompanied by much hemorrhage the anæmia is more intense. In Buchard's case, after three weeks with considerable epistaxis, the count was 557,000. In some grave cases macrocytes, microcytes, and fragmented reds are found. The color-index is reported low.

The Value of Blood Examination.—Much has lately been written of the value of blood examination. But its "value" is measured by the "practical use" which may be made of it, and not by any interesting yet "useless" information it may throw on the case. The question is a fair one, especially since vast numbers of pages of matter have been printed to prove blood counting indispensable.

The first point we wish to emphasize is that blood examination is of much greater value to the medical man than to the surgeon. The internist cannot dispense with it; the surgeon can. Some diseases are best diagnosed in this way. Among them are malaria; especially the forms without definite paroxysms and with atypical course, which pass otherwise as typhoid fever, meningitis, uræmic coma, pernicious anæmia, appendicitis, tuberculosis, dysentery, even Raynaud's disease (cases with superficial gangrene), the long list of diseases which atypical pernicious malaria may simulate, and failure to recognize which results in the unnecessary death of patient. Trypanosomiasis and infections with the Leishman-Donovan bodies can be recognized only by splenic puncture. Pernicious anæmia is quite uniformly overlooked without blood examination, and the cursory glance at a fresh blood specimen sometimes saves the patient from a course of treatment for jaundice, peripheral neuritis, or tabes, with which diagnosis patients are repeatedly admitted here. Blood examination is necessary for the diagnosis of splenomyelogenous leukæmia, and that this has a practical value is shown by the fact that the majority of our cases come to the surgical side for abdominal tumors (enlarged spleen), and are sent to us after a glance at the fresh blood. The diagnosis of lymphatic leukæmia, acute leukæmia, or pseudo-leukæmia, can be made only in this way.

For the early diagnosis of typhoid fever, measles, scarlet fever, etc., the leucocyte count is valuable, the absence of leucocytosis being much more suggestive than its presence; also in acute epidemic cerebrospinal meningitis, and various abscess formations, as of the liver or brain.

The leucocytosis is very valuable in pneumonia, especially central, and that of children and drunkards. An ever-increasing number of cases of trichinosis are recognized by the eosinophilia alone; chronic poisoning with coal-tar products was recognized in a neighboring city, notwithstanding the violent denials of the patient and her husband; various tuberculous infections are thus differentiated; the secondary anæmias due to cancer, from primary anæmia. These are only a few illustrations of the more interesting uses of blood examination.

For the surgeon, except as a differentiating diagnostician and for that he needs the blood report as much as the internist, the case is different. For him blood examination is almost synonymous with leucocyte counting. We can well appreciate the position of those men to whom blood-work is a novelty, who were successful surgeons before its day, who pride themselves that they do not need it and, indeed, are better off without it; of the men who try to use it, have never studied it themselves but must depend on assistants to interpret results for them, and who complain of the times they have been deceived; they expect too much from it; and of those who were once the blood-counting assistants themselves, who believe and often make good their boast that they can guess from the patient's general condition what the count in his case is, and if another figure is reported demand its confirmation or are sceptical as to the assistant's skill.

The question the surgeon usually demands of a leucocyte count is "Should I operate or not?" on a suspicious case of appendicitis, typhoid perforation, etc., and for this problem the count is half a point and the interpretation of it the other half. Almost never can the count decide the question alone. Some surgeons state they value it; more disregard it. The former never consider it as more than one of many symptoms, and very seldom as important an one as is the history, the physical examination, temperature, pulse, etc., but still of some help.

In acute abdominal and pelvic cases, when the question is one of an immediate operation, leucocyte counts are not indispensable. A man who knows well the field uses the blood report when convenient, that is all. One confession of its inadequacy is the value claimed for the iodine reaction, etc.

Both the medical man and the surgeon should remember that one count is seldom enough, any more than is one temperature determination enough; it is the series that counts in diagnosis, just as it is in

following a case awaiting operation. Unfortunately, blood examination takes time; yet not as much as is sometimes thought. A good hæmoglobin estimation can be made in from five to ten minutes, a leucocyte count in fifteen.

For our American clinics the message is, less routine blood-work, but a better quality of that which is done. The examination of the fresh specimen will save a great many unnecessary routine counts. But when the blood examination is important, as it so often is in internal medicine, the work should be well done and repeatedly done; well done as regards technic, consideration of the condition of the part pricked, the hour of the last large meal, etc.; repeatedly, until the curve is determined.

MALARIA

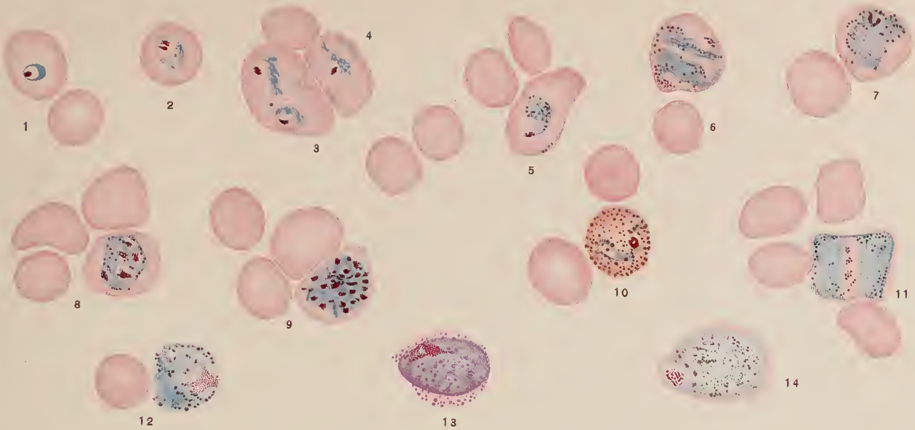
A few of the terms needing definition are the following: *Schizogone,* the asexual generation; *gametoschizont,* the sexual generation; *schizont,* or *monont,* a parasite of the asexual generation; *merozoite,* a segment (hyaline); *gamete form,* one of the sexual generation. Of the gamete forms, the *macrogamete* is the female cell; the *microgametocyte,* the parent male cell; and *microgamete* the male cell, which is one "flagellum" of the microgametocyte. *Sporogone,* the cycle in the mosquito; *vermiculus,* or *ookinet,* the motile fertilized macrogamete; *zygote, oöcyst, sporoblast,* are terms given to the spore cysts; *sporozoit,* the young sexual form which develops in the sporoblast, and which, when inoculated into the blood, becomes a hyaline.

By *pigment* is always meant the transformed hæmoglobin, or "melanin," the brown granules of which are seen in the fresh specimen, never the chromatin granules.

Hyaline always means a non-pigmented young form. A *ring form* is the shape which any young parasite may assume; it is not a "kind" of organism. *Presegmenters* are full-grown parasites the pigment of which has accumulated into masses and before segmentation appears.

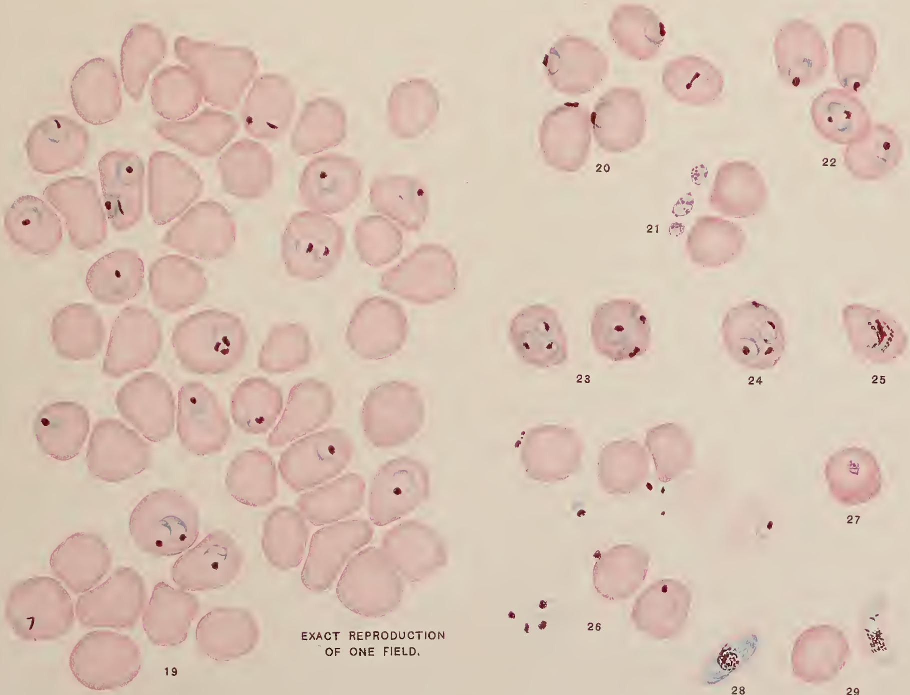
The examination of the fresh blood is easy and satisfactory. The forms can be more easily recognized in this way than in the stained specimens. On the other hand, they are more easily found in stained specimens, and when very few the Ross method should be used. While a diagnosis may be made without blood examination in typical cases, it never will be made without it in certain atypical, even pernicious, cases without suggestive history or without fever, or with typhoidal temperature.

The "Tertian" Organism; Hæmamœba vivax (Grassi); **Plasmodium vivax** (Plate IV).—This is the commonest form in Baltimore. Since the cycle extends over approximately forty-eight hours the paroxysms in the case of a single infection will occur on alternate days. The grouping is fairly definite, all the parasites undergoing their development quite in unison; the paroxysms occur during segmentation, and last from twelve to fourteen hours. In the case of a double infection there will be a paroxysm each day, "quotidian" fever, and in the blood will be seen two groups. Three groups very rarely occur, but we have seen one or two cases. The tertian hyalines (1–4) do not modify their



TERTIAN MALARIA.

QUARTAN MALARIA.



ÆSTIVO-AUTUMNAL MALARIA.

STAINED WITH HASTING'S MODIFICATION OF
ROMANOWSKI'S STAIN. ALL DRAWN TO SAME SCALE.

F. S. Lockwood.

red blood-cell host either in size, color, or contour. The parasite is small, a little over 2 microns in diameter, colorless, non-pigmented, often disk-shaped, with an undulating periphery. It makes very rapid amœboid movements and produces an extraordinary series of changes of shape and position. It also assumes the typical ring form once supposed to be characteristic of the æstivo-autumnal parasite. This ring is usually a little thicker at one point, hence the name "signet ring." In one cell may be one, two, or even five, such forms. In about twelve hours the corpuscle (6–7) will be a little larger, a little paler, but with a sharp, smooth, round margin. The organism is exceedingly amœboid, the pseudopods often many in number, and so thread-like and pale that their connections can scarcely be seen; hence the cell may seem to contain a number of disconnected globules of pigmented protoplasm. The protoplasm is so little refractive that the outline of the parasite is difficult to make out. (It is thought by some that the parasite is more distinct and sluggish after the patient has began to take quinine.) The pigment has at this age appeared in moderate amount, and consists of very fine, light-brown granules, which dance with a motion so rapid that waves in the protoplasm must be assumed. The pigment is clustered especially at the ends of the pseudopods. The untrained eye, particularly of one who has not yet learned how to light the specimen well, will see merely a swollen pale corpuscle in which dance very fine pigment granules. At the end of twenty-four hours the cell (Plate IV, 8) is somewhat larger, paler, but still round in outline. The organism now fills about one-third of the cell. It is still quite amœboid, but less actively so. The pigment has increased in amount, is a little darker, a little coarser, a little quieter, and is evenly distributed through the substance of the parasite. The nuclei of these forms can sometimes be seen in the fresh specimen as a globular body at the end of a pseudopod, and especially in the degenerated extracellulars when spread out against other cells.

During the last half of the cycle the growth is more rapid, and hence students often judge the age wrongly, considering size directly proportional to age. At forty hours the parasite (9) is full-grown. The cell is now about one and a half times the normal size. It is so pale that its outline will hardly be seen; is, in reality, nothing but a shadow. The organism is from 8 to 10 microns in diameter, is round, and so little refractive that it is practically impossible to say where the parasite leaves off and the corpuscle begins. The pigment is more abundant and is evenly distributed throughout the parasite, an important point in diagnosis, since in the quartan at this age it will be practically all in the periphery, and in the æstivo-autumnal at the centre.

The next stage is the "presegmenter." The corpuscle is now

almost or quite invisible. The pigment collects in one or more irregular clumps, the granules moving in irregular lines to form these masses. The organism is next a "segmenter" (10–17). The corpuscle is no longer seen, the organism is slightly more opaque, denser, more refractive. Refractive dots appear irregularly in the body of the protoplasm, from 15 to 20 in number, crenations are seen at the margin, and lines of separation appear around these refractive dots marking off the future segments. The segments now become more sharply defined, until finally we have a clump of fifteen or twenty discrete circular masses with a refractive dot in the centre. The clump may be irregular or form two quite concentric circles. The pigment is merely left in masses between these segments. The segmenter now seems to burst, and the young organisms spring apart. Each segment is a hyaline, and is ready for a new cell as host.

The whole cycle may occur in the peripheral blood, but the number of segmenters found will not be as large as would be supposed from the number of parasites seen a few hours previously, since so many of them have accumulated in the internal organs. A few hours after the first segment appears the chill begins.

The above is a description of a typical tertian parasite. One finds, however, some variations in this group. In one rare form, a few cases of which we meet with each year, the parasite forms more pigment than usual and in large coarse granules, but of a lighter brown color than those of the quartan or the adult æstivo-autumnal, and which form dense clusters at the ends of the pseudopods, so filling them that the granules cannot dance at all. The fine thread-like pseudopods stand out with great distinctness. The cell containing it is often not swollen but very pale, yet in one such case all the full-grown forms found were in cells from 8.5 to 13.3 microns in diameter.

Pigmented leucocytes are common (perhaps since the pigment granules are conspicuous).

The grouping does not seem to be so definite, and hence the chills are slightly longer than usual.

Extracellular Tertian Forms.—These are of two varieties, the degeneration forms and the gametocytes. The degeneration forms, or the extruded intracellulars (18–21), may in a short time after the specimen is made be the only ones seen. These are parasites which have burst from their cells and died. The organism is often seen to "run out" as if through a very fine hole. If it entirely escapes the hæmoglobin leaves the cell through the same opening, and only a shadow is left, but very often it does not, and hence we have the dumb-bell-shaped form with the constriction at the orifice. After the parasite is free in the plasma the pigment will for a time be extremely active in movement and then gradually become quiet, as the organism dies and then degenerates. It may break up into fragments, forming a string of four or five small pigmented spherical masses (20, 21), or

it may become deformed or swollen and vacuolated, the so-called "sporulating forms" which much resemble reproduction forms (23, 24).

The more interesting extracellulars (but stained specimens show that these are surrounded by the shell of a corpuscle) are the gametocytes, which correspond to the crescents of the æstivo-autumnal form, but have a less distinctive shape. Like them they can be found at all times in the blood after a few days of the infection. The macrogamete was formerly considered a cadaveric form, and was known as a "swollen extracellular." They are large organisms, pale and indistinct, some three or four times the size of a red blood-cell. In some no trace of a corpuscle can be seen, the pigment is abundant, in very coarse rods, and in very active movement. The nucleus is about 3.5 microns in diameter, and is often evident in the fresh specimen; either its outline can be seen, or its size and shape may be recognized since it is the only portion of the parasite which is not invaded by the pigment granules. The extreme vitality of these cells is astonishing, as might be expected from the fact that it is their function to continue the life of the organism in the mosquito. Recently one with particularly active granules was left by a student under his microscope in a moderately warm room. Eighteen hours later the pigment was still actively dancing. Whether these very large forms with such active pigment and quite unlike those in the stained specimens we call macrogamete forms, are the same or are fertilized forms, I do not know. The microgametocytes, smaller than the former, are from 8 to 10 microns in diameter. The pigment is in active motion, but soon forms a circle around the centre and becomes stationary. As a rule, nothing more happens. But the pigment, instead of collecting, may become even more active, as if stirred up by something moving within the cell. The margin may undulate, and the flagella, four or five in number, burst out. These flagella are the microgametes or male elements. Although the name "flagellum" is still used, it should be borne in mind that it is decidedly a misnomer. They are from two to three times the length of a red blood-cell, of regular outline, or rendered irregular by fusiform masses of protoplasm often containing pigment granules which render them more conspicuous and much easier to follow when they break loose, and wander for more than an hour through the field. After these flagella have been cast off a small cell with its pigment near the centre and quiet is all that remains. This process of flagellation is not seen in the very fresh specimen, but occurs in from fifteen to twenty minutes after the blood has been drawn, proof that it does not occur in the body, but under the stimulus probably of the lowered temperature. Normally it occurs in the mosquito's stomach.

Quartan Malaria. Hæmamœba malariæ (Plate IV).—Of this rare form

we see but one or two cases a year. The cycle of development requires seventy-two hours, hence if but one group is present the paroxysms will occur on each fourth day; if two groups, there will be two days with paroxysms, and then one free day, followed by two more paroxysms; if three, quotidian fever, providing each group is large enough numerically to cause a chill. The grouping of this parasite is even more uniform than that of the tertian, the forms being nearer of the same age, and hence the paroxysms are slightly shorter, often requiring but ten hours.

The hyalines (26) cannot be distinguished from the tertian, but may be a little later when the pigment first appears (27), since the granules are coarser, blacker, and less actively vibratory. As the parasite grows the corpuscle becomes slightly smaller and shrunken, and with an irregular crenated margin; but much deformity is rare. The protoplasm of the parasite is more refractive than of the tertian, even looks waxy, hence the outline of the pseudopods is easily seen. The parasite is definitely amœboid, but not actively so.

In twenty-four hours the cell is smaller, crenated, and brassy in color. The organism is round or oval, sometimes slightly amœboid, but very sluggishly so. It is very distinct, since refractive. The pigment is coarse, blackish-brown in color, gathered at the periphery, especially on one side. At this age all motion of the pigment granules has practically ceased. The parasite soon fills from one-third to one-half of the cell (30, 31), becomes rounder, and is non-amœboid. The cell may be shrunken, crenated, and brassy, although some may not seem in the least altered. The pigment is coarse, much blacker than the tertian, motionless, and entirely at the periphery. The protoplasm is very distinct, refractive, and waxy in appearance.

During the third day only a rim of the cell is left, and this is usually of a dark, brassy color. The organism (32–34) is now full-grown and about 7 microns in diameter, the figure usually given.

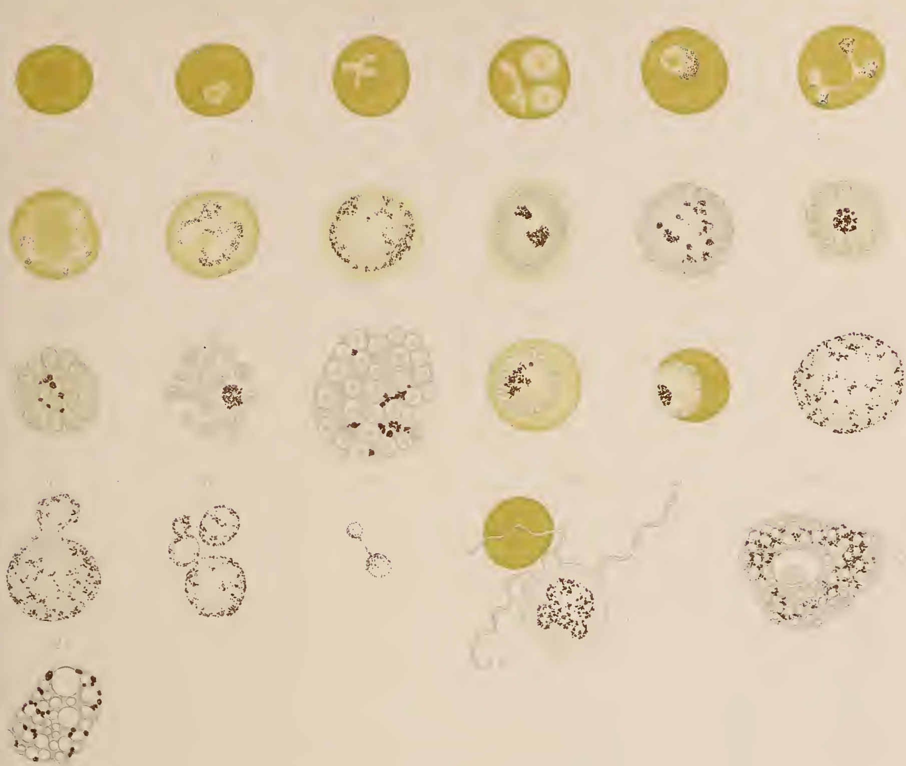
We have measured a good many quartan parasites, and, contrary to this, find that of 135 full grown and segmenters, 60 per cent. were from 7.4 to 8.1 microns in diameter, and only 18 per cent. small, from 6.2 to 7 microns. Of those two-thirds grown, 43 per cent. were in cells from 6.2 to 7 microns in diameter, the rest in cells of normal size.

At sixty hours the cell can hardly be seen. The organism is round or elliptical and motionless (35). The coarse dark pigment is all at the periphery.

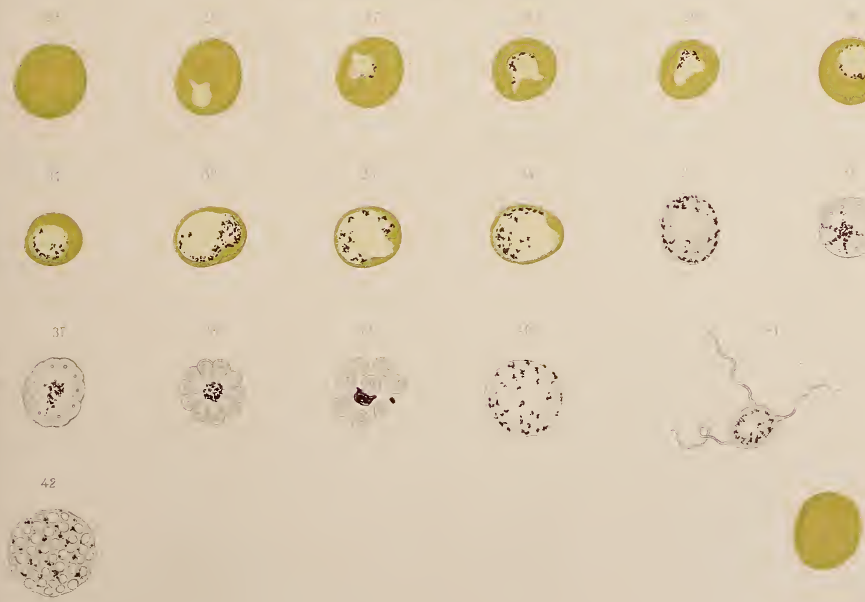
The pigment now flows to the centre in definite streams along radial channels, thus giving a beautiful wheel-like picture (36), the rows of pigment granules forming the spokes, then finally collects in a single clump at the centre. These are the presegmenters. The seg-

PLATE IV.

The Parasite of Tertian Fever



The Parasite of Quartan Fever



menters are among the most beautiful things seen under the microscope. The organism becomes opaque and very waxy; refractive dots appear in a single regular circle around the periphery; crenations of the border appear with these dots as their centre; lines of division start from these and run to the centre, forming from six to twelve rays like the petals of a flower, hence the names "daisy," "marguerite," or "rosette" form (37, 38). These segments then separate as in the tertian. The whole cycle of the quartan occurs in the peripheral blood, hence one finds about as many segmenters as the number of the full-grown parasites would lead one to expect.

The gamete forms (40, 41) are very seldom seen. They are similar but somewhat smaller than the tertian. Flagellation occurs in the same way. The extracellular degenerate forms are found, although the parasite keeps in the cell much better than does the tertian.

In review, the differences between the tertian and the quartan may be stated as follows: The cycle of the quartan is seventy-two instead of forty-eight hours. This organism is, throughout its entire history, smaller, more refractive, less amœboid, its pigment is coarser, blacker, less vibratory than the tertian, and keeps a peripheral position. The corpuscle is shrunken, crenated, and brassy. The presegmenter and segmenter forms of the quartan are perfectly distinctive, since they are of so geometrically regular forms. The number of the segments is small, from 6 to 12; and, lastly, more of the segmenting forms are found in the peripheral blood.

Æstivo-autumnal. Plasmodium præcox. Hæmatozoon falciparium (Plate V).—This is a common form, particularly in the Tropics, and the most dangerous of the three. In the fresh infection the grouping is quite definite, but soon the members of a group lose their unison, and hence are found in the internal organs of all ages at once. For this reason what was first an intermittent fever becomes more and more continuous, until finally the temperature may resemble that of typhoid fever. The duration of the cycle is rather uncertain. Dr. Thayer considers that while usually of about forty-eight hours, it may vary from twenty-four to perhaps seventy-two.

All students, it is said, "pass through the stage" of dividing this form into "benign," "malignant," "pigmented," "non-pigmented," etc., varieties, but most recover, especially those who follow the splenic blood carefully. Some separate it into the "malignant quotidian" and the "malignant tertian," the latter similar in form to the "benign tertian" (ordinary tertian), except smaller, from one-third to one-half the size of red cells, and with from 8 to 12 segments. The "malignant quotidian" is from one-third to one-fifth the size of red cells, "often unpigmented" and with from 6 to 8 spores. In this locality we are often struck by the great difference in the æstivo-autumnal parasites, both clinically and morphologically, especially as regards the amount of pigment and the presence in the circulation of the adult much-pigmented forms, but Dr. Thayer thinks such division not justified as yet.

The hyalines are similar to those of the tertian and the quartan, perhaps are slightly smaller, but they assume the signet-ring form much more commonly and hold it longer. Then they are very refractive, hence easily seen, but may at any time lose this refractivity and become amœboid exactly as does the tertian. In a severe infection even five rings may occupy one cell.

As the parasite (7–12) grows a very slight amount of pigment appears, usually but one or two granules, and so fine that they are very easily overlooked. These are motionless as a rule, although sometimes slightly dancing, and are seen at the periphery of the parasite or at the inner edge of the biconcavity. The cell is commonly very much injured, shrunken, crenated, and brassy, even when the parasite is very young. This form injures the cell most, also many corpuscles which do not contain parasites, yet some infected cells look normal. The parasite at this stage fills about one-fifth of the cell. As a rule the infected cells now disappear from the peripheral circulation, perhaps since the injured cells are treated as foreign bodies, hence are filtered out, and to study their further development the spleen must be punctured. The suddenness of their departure is quite surprising, as well as exasperating to a demonstrator, since in two hours a large brood may disappear. Hence it is that if no crescents are present the diagnosis will be uncertain unless repeated examinations of the blood are made. In some cases, however, all ages of this parasite may be found in the peripheral blood. In these cases and in the blood obtained by splenic puncture, the pigment is seen to increase considerably in amount, and to be in rather coarse, dark granules, or remains scanty, while in some scarcely any seems to form. Those parasites with much coarse black pigment it is impossible to tell from quartan forms, and this mistake is frequently made. The more malignant the parasite the fewer older forms are seen in the peripheral blood, and according to some the less pigment is formed; the pernicious cases always have abundant young parasites in the peripheral blood. In some cases the hæmoglobin seems to gather around the parasite, leaving an almost colorless ring at the periphery of the red cell (13). In the internal organs the whole cycle seems to occur inside of large macrophages. The parasite grows to about one-half the size of the cell (5 microns). When full-grown the pigment is all in the centre (15–20), never diffusely scattered, and never peripheral. The protoplasm is waxy. This form is very characteristic. Although rarely seen in the circulation, in two cases recently during the class demonstration we found several beautiful full-grown and one segmenting form (see Fig. 93). The segmenters vary in size from 2.5 to 5 microns in diameter. The process of segmentation (21–24) is similar to that of the tertian, the waxy

PLATE V.

opaque organism breaking up irregularly into fifteen or sixteen very small segments. Very few degenerated extracellulars are found.

Crescents and Ovoids.—These very characteristic forms of the æstivo-autumnal parasite are found in the internal organs from about the fifth day of a fresh infection, and appear in the peripheral blood on about the seventh day. The crescents (29) are slightly longer than the red blood-cells, sometimes of a beautiful crescentic shape with rounded ends; others are somewhat irregular. They are very refractive, with a double contour, and usually present a fringe of the degenerated red blood-cell which in the concavity is somewhat more abundant and forms the so-called "bib." The pigment is considerable in amount, clustered at the centre of the crescent either as a confused mass, a sheaf, or a ring. The granules are coarse and usually rod-shaped. While watching the parasite it may lose its crescentic shape and become first oval (ovoids, 30–33), then circular (34–36), or it may resume the crescentic shape. Around the circular there is no trace of the corpuscle left; its protoplasm is much less refractive than the crescent. Two forms of the circulars have been described in the fresh blood, the macrogamete and the microgametocyte (see page 602). The former may flagellate, and fertilization has been seen by several observers, first of all by MacCallum [98] and then others of this hospital, and more recently by Moore, *et al.*[99] Vacuolation and fragmentation of these sexual forms are not rare (Plate V, 37).

The phagocytes are well studied in this form of malaria; in fact, pigmented leucocytes are as valuable in diagnosis as is the parasite itself. These are large mononuclears especially, polymorphonuclear neutrophiles, and macrophages (see page 559, and Fig. 93). Pigmented macrophages are seen only in severe cases. In these phagocytic cells are found free pigment granules, or masses of pigment, or parasites, especially segmenters and flagellates. In the tertian and quartan they occur just after a chill, but in the æstivo-autumnal at any time. The large macrophages especially contain organisms, even those within cells. Some of these macrophages are necrotic.

The malarial pigment is black, "melanin," and iron cannot be demonstrated in it.

The Cycle within the Mosquito.—This cycle has been followed by several observers in the case of Plasmodium præcox. Some of the crescents of the blood contained within the stomach of the mosquito are seen to flagellate in the same way as on the stage of the microscope, and probably due to the same stimulus, that is the lowering of temperature, and to fertilize the female circulars, which also were crescents. This occurs in from 1 to 1.5 hours after the mosquito has bitten. During the flagellation of the microgametocyte the macrogamete ripens by casting off karyosomes, polar bodies consisting of chromatin, and projects a

[98] Johns Hopkins Hosp. Bull., November, 1897.
[99] Johns Hopkins Hosp. Bull., October, 1902.

slight mound, through which the free flagellum has been seen to enter. The nuclear material of the macrogamete and the microgamete then unite. The cell remains naked and assumes a motile spindle form called the "vermiculus." Its size varies from 20 microns up, and is to be found in from forty to forty-eight hours after the blood has been ingested. This motile form in the case of malaria has been found only in the contents of the mosquito's stomach. This vermiculus

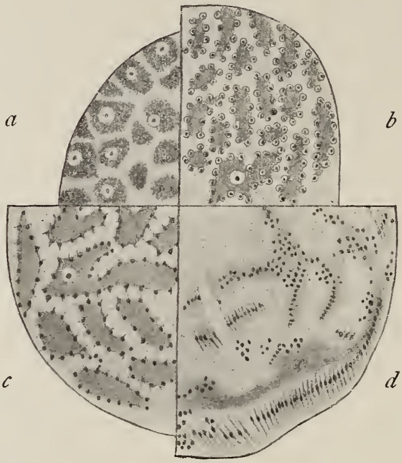


FIG. 116.—Various stages of the development of Plasmodium præcox in the mosquito's stomach. *a*, In four to four and a half days after the bite; *b*, *c*, five to six days; *d*, eight days (Plasmodium vivax). (From Braun.)

actively bores its way through the epithelial cells of the intestinal wall, and becomes encysted between the intestinal epithelium and the elastic layer, the "tunica elastico-muscularis," which forms the membrane of the oöcyst. This oöcyst now increases in size. The nucleus divides rapidly. As the cyst grows it bulges outward from the intestinal wall, forming a pendulous tumor into the body cavity (see Fig. 117). These vary from 4.5 to 30, or even 60, and as high as 90 microns in diameter. This

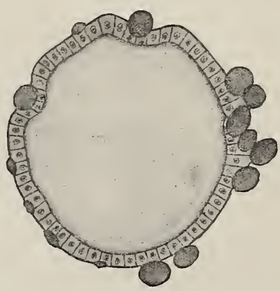


FIG. 117.—The intestine of an infected mosquito with oöcysts attached. (From Braun.)

stage is called the "medium zygote," or the "medium sporoblast," and is conspicuous because of the amount of pigment. There may be 200 such tumors attached to the intestine of the mosquito. The protoplasm now gathers around the divided nuclei (Fig. 116, *a*), a process analogous to the sporoblast formation of the coccydia except that here the separation is less perfect, the daughter cysts being connected by bridges of protoplasm. It is now known as a "large zygote"

or a "large sporoblast." In each of these divisions the nucleus divides into great numbers (*b*, *c*), the daughter nuclei remaining on the surface of the various daughter cysts. The protoplasm collects around each, first forming spherical cells, which then elongate into threads lying parallel in masses over the residue of the sporoblasts. These threads are called "sporozoits." Their nucleus also becomes elongated. The final length of these sporozoits is about 14 microns, and the width about 1 micron. Their protoplasm is thick, homogeneous, and very refractive. All sporozoits of one oöcyst ripen at about the same time; they may be present even to the number of 10,000 in some oöcysts, while others contain but a few hundreds. When ripe the oöcyst bursts into the body cavity, the sporozoits wander free, but, as if directed by some positive chemotactic influence, finally collect in the salivary glands. They are motile, moving by a bending gliding movement. Inoculated by the mosquito's bite into the blood-vessel of man, they attach themselves to and finally penetrate into the red blood-cells, a process actually observed by Schaudinn. They are said to stay for some time on the surface of the cell before penetrating, and it is said that if quinine is now given they will drop off from the corpuscle. This may explain the opinion of several recent writers who insist that the hyaline and older forms are always on and not within the corpuscle. As a rule the first chill will come on about the eighth or twelfth day after the mosquito bite, although, of course, it will depend on the number and the

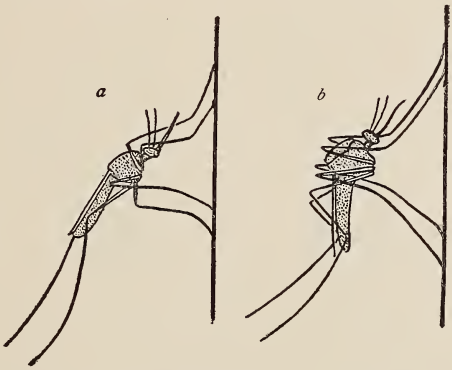


FIG. 118.—Attitude of mosquitoes on wall. *a*, Anopheles; *b*, Culex.

virulence of the parasites introduced into the circulation. Since some mosquitoes contain fully 200 of these oöcysts (of course not all of the same age), and some of these contain 10,000 or more sporozoits, the number of the hyalines injected by one bite may be considerable.

For the tertian the optimum temperature for this cycle is 28° to 30° C., and the time eight days; below 17° to 20° C. there is no development. The quartan form can develop at a slightly lower temperature.

The Anopheles group of mosquitoes is the only one as yet shown to be the host of the malarial organism. For a full description of these insects the reader is referred to various books.[100]

The Anopheles genus may easily be recognized by its attitude on a wall, since (see Fig. 118, *a*) its body is in a straight line with head and proboscis, and at an angle with the wall, the "awl shape," while Culex (*b*) sits "hunch-backed," its body parallel to the wall, its proboscis at an angle of forty-five degrees with its body. The genera are separated by the relative length of their probosces and palpi (see Fig. 119). Of the Anopheles female these are of equal length and scaled, while of the Culex, Stegomyia, and Tæmiorhyncus females the palpi are short and insignificant. It is only the female Anopheles which bites. The wings

[100] Stevens and Christophers, "Malaria of the Tropics," 1905; Nuttall and Shipley, Jour. of Hygiene, vol. i., Nos. 1 and 4; vol. iii., No. 2.

of Anopheles alone are spotted, as a rule. Anopheles usually holds its hind pair of legs stretched out and oscillating in the air.

Its egg and larva are characteristic: the former, from its boat-like shape and lateral air-cell floats; the latter, from its attitude in the water, lying parallel with and just below the surface.

Stained Specimens.—The technique is fairly simple. Very thin smears must be made (see page 418); these are stained by any one of the various polychrome methylene blue-eosin mixtures (see page 425). The fresher the smear when stained the better the preparation.

Ross has recently described a method which is of the greatest value when only a few parasites are present. A very thick drop of blood is placed on the slide and spread over an area equal in size to a ten-cent piece. It is then dried thoroughly in the air. The slide is then covered with water in order to remove the hæmoglobin. Care should be taken that the washing be not too vigorous, else the fresh

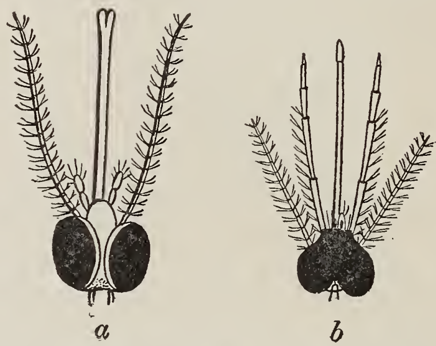


FIG. 119.—Heads of mosquitoes. *a*, Culex; *b*, Anopheles.

blood which has not been fixed will be washed off. The specimen is then stained in the usual manner. In such a specimen parasites appear numerous, when in ordinary smears scarcely one is found.

TERTIAN (Plate III, 1–14).—The youngest hyalines consist of a mass of blue protoplasm and a clump of carmine-violet stained chromatin. They are 2 to 3 microns in diameter. Soon the "achromatic zone" appears, the "vesicular part" of the nucleus, which may be the largest part of the parasite. The protoplasm now often forms a wide crescentic ring surrounding this, with the chromatin mass between the tips of its horns, but often not quite touched by them. A "milk-white zone" of Gautier often surrounds the chromatin mass, but is not present at all ages, nor in all of the same age. To just what part of this structure the term nucleus shall be applied is disputed. Stephens and Christophers, as well as many previous observers, use the term for the chromatin mass alone, while others include the chromatin mass and milk-white zone, and others also the much larger achromatic zone.

At this point should be emphasized the necessity of seeing distinctly the blue protoplasm and the red chromatin to recognize the malarial hyaline. This is necessary, since so many other structures can look almost like a hyaline, as, for instance, certain degenerations of the red blood-cells, and particularly platelets on the cells (Plate III, 21, 27). These or any other structure on the cell are always surrounded by a colorless zone, probably due to the pressure which they exert on the corpuscle, squeezing the hæmoglobin from their vicinity. It is of interest that in the case of malarial parasites this is not usually the case, but the hæmoglobin is in direct contact with the parasite, good evidence, says Ross, that the hyaline is intracellular and not adherent to the surface of the cell. (Argutinsky, Stephens and Christophers, and others.)

At the end of twenty-four hours there has been an increase in the amount of protoplasm, the achromatic zone is a little larger, but the chromatin is the same in amount, although now in a more irregular nodular mass. The milk-white zone is seen in some. Some forms have two or more such definite nuclei.[101] The nucleus of this parasite is not perfectly specialized but is rudimentary, the nuclear material being scattered in the cell or collected in one or more masses. In the full-grown the chromatin breaks up into a cluster of fine granules occupying a large achromatic zone, both of which just before segmentation seem entirely to disappear. It then reappears in fine granules arranged in strands and masses throughout the protoplasm. These congregate into four or five clusters and then separate into from fifteen to twenty dense round masses. Achromatic zones now appear around each of these masses, the protoplasm collects around them as a centre, and the segments separate. For a description with much more complex details, which tries to bring this division into the same class as all nuclear division, see Argutinsky.[102] The pigment at the beginning of segmentation is pushed to the periphery, and after segmentation is complete collects in one or two masses near the centre. It will be remembered that in the fresh specimen at the time the pigment went to the centre there was no evidence of the separation into segments, hence segmentation is a process which is really complete before there is any sign of it in the fresh specimen. It is claimed by many that in the stained specimen the gamete generation can be followed from the hyaline form onward. According to Stephens and Christophers the young gamete is characterized by the position of the chromatin, it lying in the centre of the vacuole instead of at the edge as is the rule in the asexual forms. During the cycle the cell is in

[101] For evidence of conjugation, see Ewing, J. H. H. Bull., 1900, and Clinical Pathology of the Blood, 1903.

[102] Arch. f. mikr. Anat. und Entwicklungsges., 1901, Bd. 59, p. 315.

some cases filled with basophile granules (see page 446), "Plehn's karyochromatophilic granules," "Schügner's granules" (Plate III, 10, 13). Bignami and Bastianelli claim that the division of chromatin into fine granules marks the gamete even in the hyaline stage, but Lazear doubts this, since the pigment always just before segmentation divides into fine granules.

The full-grown macrogamete (Plate III, 14) contains an abundance of protoplasm which stains a deep blue, and a small amount of chromatin in a compact mass which is peripherally placed and surrounded by a thin vacuole-like area, this nucleus occupying about one-tenth the cell. The pigment is uniformly distributed. The remains of the corpuscle often cannot be seen. In the microgametocytes (Plate III, 11) the chromatin is more voluminous, looser, centrally placed in a large achromatic zone. It is in a band arranged as a knot or skein, its thread-like nature always evident. The parasite fills about two-thirds of the cell; its protoplasm is in a ring around the nucleus. It stains a grayish-green or grayish-red color, and not at all the blue of the female form, hence the pigment is easily seen.

Quartan (Plate III, 15–18).—The structure of the quartan resembles that of the tertian, but in the hyaline the chromatin mass is less dense, is, in fact, an irregular clump of granules, and in the older forms is in a cluster of fine granules without a distinct achromatic zone, hence often hard and sometimes impossible to see. The parasite is usually a band across the cell.

Æstivo-autumnal (Plate III, 19–29).—In the hyalines the chromatin is in from one to three masses or filaments. The protoplasm is scantier than in the other forms and remains so throughout the cycle. Characteristic of this form at a later stage is the large oval ring of protoplasm with a thicker layer opposite the chromatin mass. The young gamete forms are characteristic (Maurer). They are accurately spherical, being a ring of the same thickness all the way around. The nucleus forms a portion of the ring, but this does not project as in the schizonts, and the red blood-cell usually presents no coarse stippling (Maurer). Of the crescents, the male form has its chromatin in a loose net-work which occupies the most of the cell, comparatively little blue staining protoplasm, and the pigment scattered throughout its body. This crescent is somewhat kidney-shaped, is shorter and broader than is the female form. The female crescent is quite long and narrow, its chromatin is more compact and more or less centrally placed, there is much more blue staining protoplasm, and the pigment is in a ring around the nucleus or in a clump near the centre.

There are also two types of circular bodies, the microgametocyte, smaller than the red, perfectly spherical, with chromatin in the centre in a large irregular mass like a tangled thread, later in four or

five dense masses near the periphery, which then are extruded as the flagella (or microgametes). Sometimes a thin bluish envelope of protoplasm can be stained enveloping the chromatin thread of the flagellum. The macrogamete is two or three times as large as this, often of triangular shape, with abundant blue protoplasm; the chromatin is in a single mass at the periphery and surrounded by a circle of pigment. In the stained specimens (especially in sections cut in paraffin) can be seen the projection of the chromatin mass and part of the protoplasm apparently from the surface of the cell, hence the belief (Argutinsky, Stephens and Christophers) that it at all stages rests on the cell, not in it (Plate III, 20). Study of the fresh blood, by far the more important, noting especially the way they burst through a fine opening, their amœboid phenomena, etc., shows conclusively, we think, that they are intracellular.

The following points may be emphasized: In the case of tertian and quartan malaria, organisms and chills are not synonymous. The infection must reach a certain degree (250,000,000 organisms, Ross) before chills begin. Because no parasites are found may not rule out malaria, especially if the patient has been taking quinine. Fevers with long intervals are thus explained, very many parasites being killed off at each chill, hence some delay before enough have reaccumulated to cause a second chill.

In a case of fever to find a few crescents does not mean necessarily that the fever is malaria, since these gamete forms may persist for months after the asexual cycle has stopped. In case hyalines also are found the diagnosis is justifiable, especially if the fever yields promptly to quinine. The asexual cycle is the "febrile cycle." The sexual has no influence over this host, except that it may again start up the asexual cycle, explaining relapses in early spring and especially those occurring after an accident or a surgical operation even two years after any chance of reinfection has passed. Not all the members of the same tertian or quartan group are of exactly the same size or age, and the segmentation continues through at least twelve or fourteen hours. This is fortunate, since, did they segment more in unison, hæmoglobinuria would probably be more common (as analogue, see Texas fever of cattle). The size of the segmenters varies so much that it is supposed that when the majority begin to segment all the others not quite mature as yet are drawn into a "precocious segmentation" (Plate IV, 16, 17). This keeps the groups at an almost equal age, for otherwise these younger forms would disturb the grouping to the degree which occurs in æstivo-autumnal malaria. It also may explain the sudden appearance of a second group in a previously single infection, a few of the forms being so young that they cannot be drawn into this precocious segmentation.

The conditions on a slide under the microscope are more like those in the mosquito's stomach than in the circulating blood, hence many changes (*e.g.*, flagellation, the cadaveric forms) must not be considered as occurring in the human host.

There is a remarkable periodicity in the cycle which we do not understand; among other illustrations, the tendency to flagellate. In some cases used for class demonstration so many flagellated forms will be found that all the students can study the process; in other cases with even more sexual forms not one will flagellate.

The distribution of parasites in the body is remarkable. The æstivo-autumnal lives for the most part in the spleen, liver, and bone marrow; the same to a less degree is true of the tertian. But it is their accumulation in other organs which is important; in the brain and medulla causing thrombi, hence paralyses,

transient aphasias, mental symptoms, even sudden death; and in the mucosa of the gastro-intestinal tract causing even necrosis and sloughing, hence severe vomiting and diarrhœa.

Whether the virulence of the infection depends on the number of parasites or not is hard to answer; it certainly does not on the number in the peripheral blood, although pernicious cases have usually many organisms visible.

Trypanosomiasis.—This most interesting disease in man ("sleeping sickness"), which has recently attracted so much attention, is now considered due to an actively motile fish-shaped flagellate, Trypanosoma gambiense (Plate II, 21), which can be seen in the blood-plasma, moving with a screw-like motion among the red blood-cells which it scarcely disturbs. It is from two to three times as long as a red blood-corpuscle (18 to 25 microns long, 2 to 2.5 microns wide), with one flagellum anteriorly and an undulating membrane which extends the whole length.

The parasite is to be searched for in the fresh blood specimens with a medium magnification. There are present sometimes many, generally few. They vary much in numbers, often being absent for long periods, even a month or more, and then reappearing in force, even 70 to a cover-slip specimen. The symptoms seem to bear no relation to the number of parasites in the peripheral blood; it may be necessary to centrifugalize to find any. They can most surely be found by puncturing the cervical lymph glands, and are easy to find in the fluid of œdematous areas. Inoculation experiments may be necessary. Stained with a polychrome-methylene blue and eosin mixture they have a rather large red nucleus at about the middle, a centrosome staining intensely in a vacuole-like area very near the blunt posterior end, and a red line of chromatin running down the edge of the undulating membrane and terminating in the red flagellum. The protoplasm of the body takes a blue stain. Various involution forms will, of course, soon be seen in a fresh specimen. The parasite contains no pigment and hence must live on the plasma. It multiplies by longitudinal fission.

For a long time it has been well recognized that this organism was a common and harmless parasite in the blood of fish, amphibians, birds, and rats, and an important cause of disease among horses, cattle, and other domesticated animals in India, Africa especially, and South America. The disease has borne several names. The "tsetse fly disease" of Central Africa caused by Trypanosoma brucei, is usually fatal to almost all domestic animals, especially the horse, the mule, and the dog, less so for cattle and still less so for the ass, least for sheep and goats. Man was, however, supposed to be immune. It is communicated by a fly, Glossina morsitans. Flies seem to carry it mechanically, and to play no part in its life history.

The "surra," of India, a disease which attacks horses and camels

especially, is caused by a parasite discovered in 1881 by Evans, which differs in no way from Trypanosoma brucei. The same may perhaps be true of the parasite of "mal de Caderas" of Central and South America, which attacks especially horses.

The parasite was first discovered in man by Dutton in 1902 in the blood, and in the cerebrospinal fluid of a case of sleeping sickness by Castellani, but it was Bruce who first recognized its pathogenic importance in man. This disease is communicated by Glossina palpalis.

Of eighty persons in good health in Uganda, Bruce found the parasite in the blood of twenty-three, but many of these have since died. The parasite may thus be found in men apparently normal for some time, but the present opinion is that it is sooner or later fatal. The symptoms are somewhat like those of malaria. It is a disease which can take a rather acute course, but as a rule is exceedingly chronic, running for years, yet uniformly fatal when the parasite invades the cerebrospinal fluid which seems to be the common or perhaps unfailing result. It is accompanied by an irregular temperature often with intermissions, by multiple erythema, moderate anæmia, marked emaciation, loss of strength, localized œdema of face, trunk, and legs, enlarged spleen, and swelling of the lymph-glands, especially those of the posterior cervical region. Later, the so-called "sleeping sickness" begins, which is due to the presence of the parasite in the cerebrospinal fluid, and found there in practically every case (Bruce).

This fluid should be centrifugalized gently for fully five minutes. It can then be poured off to the last drop into another tube and the sediment examined under a well vaselined cover-glass. If centrifugalized too violently their motility will be less, and they may be mutilated by the weight of the sediment. The fluid should be centrifugalized two or even three times, for none may be found in the first sediment. Not many are present.

The parasite of man, Trypanosoma gambiense, can in no way be distinguished from that of the tsetse or surra, either morphologically or pathogenically.

There are two other forms of trypanosoma which are easily distinguished from that of man, and which occur quite commonly. The one is Trypanosoma theileri, which is pathogenic for cattle alone,—it is a parasite from two to three times as long as the human form,—and the trypanosoma of rats, which is morphologically characteristic, since the posterior end is long drawn out and pointed, the centrosome is not near the end, but at the juncture of the posterior and the middle thirds. It can be easily distinguished from the other trypanosomata, even when they coexist in the blood. It occurs in about 10 to 30 per cent. of rats investigated in some regions, in others in even 90 per cent.

For a recent discussion of the whole subject the reader is referred to the report by Musgrave and Clegg.[103] A recent good brief review is by Koch.[104]

Piroplasmosis. Infection with the Leishman-Donovan Bodies.—The Leishman-Donovan bodies (see Fig. 120) are small, oval, round, or oat-shaped bodies, from 2.5 to 3.5 microns in diameter. They have a definite cell outline and contain two chromatin masses, a larger one, the " nucleus," almost round or oval which stains faintly, and a smaller, bacillus-shaped " centrosome," which stains deeply, and which is directed either at right angles or nearly so to the axis of the nucleus. These two bodies are both in the long axis of the cell, the larger on the periphery. Many are vacuolated. The outline of the cell cannot always be seen, but these two masses thus arranged are distinctive. They are easily stained by the various polychrome methylene blue-eosin mixtures. They are best studied with the highest powers, the oil lens and V ocular.

They are not found in the circulating blood, except a few intracellulars in two fatal cases, but easily in that from splenic puncture, and in the granulation tissue snipped off from the ulcers with scissors and crushed thin on the slide. At autopsy many are found in the mesenteric lymph-glands, bone-marrow, and liver.

Some lie free but most seem to be intracellular, one or two in a leucocyte (?), from one to twelve in endothelial or splenic cells, some, even hundreds in large masses, in macrophages(?). These masses are variously interpreted. If cells, they are badly degenerated. Ross considers them to be a "matrix" in which the organisms lie, and that none are intracellular, and Manson regards such masses as zoöglia.

Their division may be followed. It begins in the larger chromatin mass and ends in the smaller which may begin to divide after the fragments of the larger are widely separated.

This parasite is supposed to be the cause of some cases of the chronic " malarial" cachexia of the Tropics, dam-dam fever, kala azar, tropical splenomegaly; of the tropical ulcer, Delhi boil, Aleppo button, Scinde sore, oriental sore, etc. It is a filth disease. In the Tropics it promises to prove even more important than the malarial organism. Clinically there are great enlargement of the spleen, emaciation, irregular fever, various abdominal symptoms, and cutaneous hemorrhages and ulcerations.

Donovan reports 72 cases,[105] with a mortality of 30.55 per cent.

The blood features are a moderate anæmia, from 2,000,000 to 4,000,000, and leucopenia, with a relative and absolute increase of the large mononuclears. The average leucocyte count is about

[103] Biological Lab. Department of the Interior, Bureau of Government Laboratories, 1903.

[104] Deutsches med. Wochenschr., 1904, No. 47.

[105] Lancet, September 10, 1904.

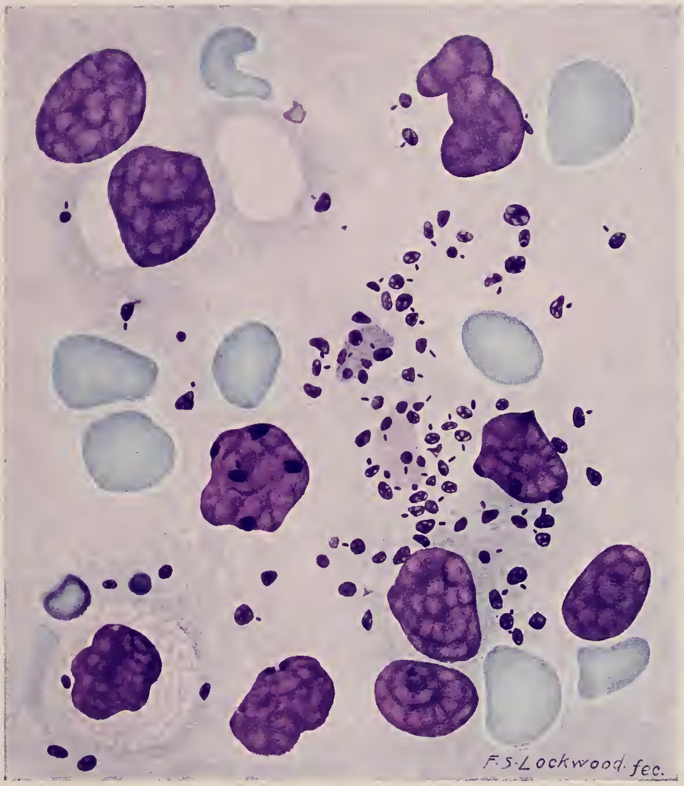


FIG. 120.—Leishman-Donovan bodies. From splenic puncture. × 1200.

2000. In a case of Neave the large mononuclears were 67 per cent. (total count 3000); pmn. n., 20 per cent.; sm. monos., 11 per cent.; eosin., 1 per cent.; myelocytes, 1 per cent. The patient was an eight-year-old boy. In most cases the formula is more nearly normal.

These parasites were first described in 1900 by Leishman as degenerated trypanosomes, an idea which is now held by some. Whether related or not, it is agreed that these may show flagellated forms, and that degenerated trypanosomes can assume a form similar to this, but those interested refuse to find any relationship.

Filariasis.—Of the various forms described in human blood, that most common is Filaria bancrofti (F. nocturna). These embryos are from 270 to 340 microns long, and from 7 to 11 broad (see Fig. 121). They are enclosed in a sheath which is considerably longer than the parasite. The anterior end of the worm is abruptly rounded, with six-tipped prepuce and sharp fang; the posterior tapers off for two-fifths of its length. It has a granular median axis. At

FIG. 121.—Filaria bancrofti. × 50.

first their movement is progressive, but they seem soon to become attached to the glass at their anterior end, and there they remain, lashing the surrounding corpuscles for days. These embryos appear in the circulation towards evening, their number gradually rising to a maximum at about midnight, then diminishing toward dawn. During the day they are in the internal organs, especially the lungs.

Lothrop and Pratt [106] charted hourly the numbers counted, and found at midnight 2100 per cubic centimetre.

The adults lie in the lymphatics, where they obstruct the lymph flow, causing lymph-scrotum, elephantiasis, occlusion of the thoracic duct, and various other lymph tumors. This obstruction is also attributed to the eggs, which are too wide to pass through capillaries. This is the chief cause of hæmatochyluria. The female is 85 to 150 mm. long, with a distinct neck, a head with simple minute terminal mouth, a plain cylindrical body covered by a striated cuticle, and which tapers to the neck and tail. The tail ends bluntly and has a small depression surrounded by two lips. The anus is a ventral opening on the summit of a trilobed papilla.

[106] Am. Jour. Med. Sci., November, 1900.

The ova are 25 to 38 microns long by 15 microns broad. The females are generally vivaparous, but may discharge the eggs. The embryos reach the general circulation through the thoracic duct.

The male is 80 mm. long without neck, and a tendril-like tail rolled up in one or two spirals. The œsophagus is thick walled. The cloaca is ventral, with four pre-anal and four post-anal papillæ and two spicules.

The intermediate hosts are some varieties of Culex and Anopheles mosquitoes. About an hour after the bite the embryos in the mosquito's stomach cast their sheath. Some die, but others actively bore their way through the intestinal wall to the muscles, where they rest. During the next two or three days the embryo becomes larger and the alimentary tract develops. On the seventh day the worm is 1.5 mm. long and perfectly developed. It now actively travels to the head and takes its position in the labium, whence it enters its new host during the biting by piercing the delicate membrane of the end of the proboscis. It takes an infection by even hundreds of these adult forms to cause a very severe case, and it may be years before any symptoms begin.

The clinical symptoms, in addition to the various lymph tumors, are anæmia, enlarged spleen, and fever. In any case of lymph tumor, elephantiasis, hæmatochyluria, the blood should be examined. These cases are usually admitted to the surgical side, and an interesting number have been operated on for inguinal hernia, the lymph-scrotum being thus interpreted. Probably there are a good many cases in this country, judging from the number recently found in quite widely distant cities.

It occurs endemic in the Tropics. In the Fiji Islands as much as 25 per cent., and in the Friendly Islands even 32 per cent., of the inhabitants are said to be infected with this disease, also called "craw-craw," or the "sleeping disease."

The blood should be examined late at night. A very thick fresh specimen is made and examined with the low power. These worms cannot be overlooked. Their motion will continue for even a week in a well-sealed specimen.

The *hæmatochyluria* is due to rupture of the varicosed lymph-vessels of the bladder, these forming much of the collateral circulation which compensates for an occluded thoracic duct. The attacks may occur for even eighteen years, each being weeks or months long and separated by intervals of months or years. They come on spontaneously or following exertion, excitement, etc. The onset is with pain and fever. The sequence is, hæmaturia, hæmatochyluria, chyluria. In the urine are found embryos. The urine contains most blood and embryos in the early morning, most chyle after a rich meal (even 3.8 per cent. fat). (For the blood formula, see page 501.)

Filaria diurna (the embryos) differs little from Filaria nocturna (bancrofti), except that it remains in the circulation only during the day, and the adult form is not yet known (Filaria loa of the subcutaneous areolar tissue?). The granular axis fails.

Filaria perstans.—These embryos are about 200 microns long and 4 to 5 in width, without a sheath, and with very active, progressive, as well as lashing motion. They remain in the circulation day and night. The body tapers for its posterior two thirds; it has a slightly bulbous tail.

The adult is situated in the retroperitoneal tissue.

Other forms described in man are Filaria ozzardi (embryos small, 170 to 200 microns long, without sheath and with sharp tail, no periodicity, its adult in the sub-

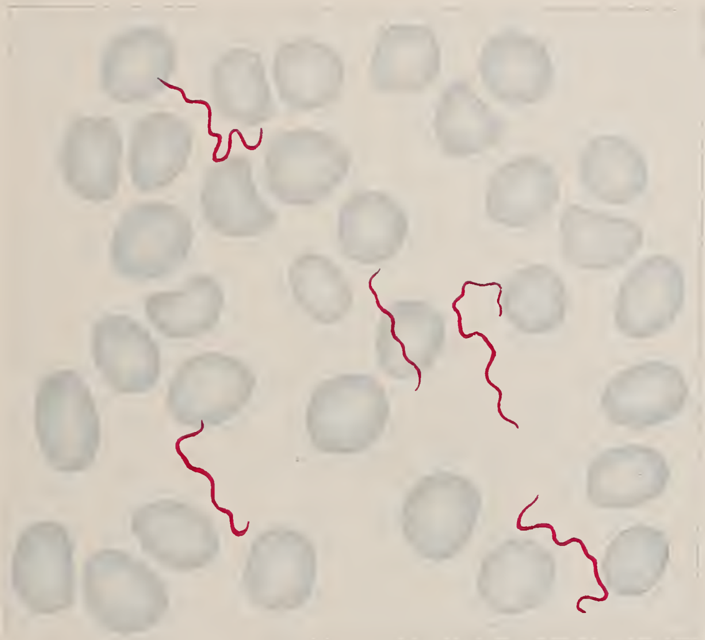


FIG. 122.—The spirochæte of relapsing fever. × 1200.

peritoneal tissue); Filaria demarquai (embryos 200 microns long, sheathless, and sharp-tailed, with cephalic armature, no periodicity, adult doubtful); Filaria megalhæsi, Filaria gigas, and Filaria loa.

Relapsing Fever. Famine Fever.—The spirochæte of Obermeyer (see Fig. 122) is an organism usually curled like a corkscrew, from 25 to 40 microns long and about 1 in width. No further structure is evident. It takes a deep chromatin stain. It occurs in the blood only during the febrile paroxysms of this disease. Nothing more is known as to its life history, but it is certainly a filth disease. It is actively motile, with a rapid, delicate, wavy motion, stretching and collapsing like a coiled spring, and also moving slowly along among the corpuscles, but seeming not to disturb them much.

They appear one or two days before the paroxysm, at first few, then in great numbers, sometimes single, some twisted into snarls, many within leucocytes, at the end they disappear until the next paroxysm. The fever continues about six days; the intervals are of about the same length.

The "spores" seen in the plasma during the intervals are of doubtful significance (hæmokonien granules?). The organisms can be seen to multiply in the specimen, also to die and to break up.

CHAPTER VI

EXAMINATION OF VARIOUS FLUIDS

AMONG these are the plasma, serum, lymph, the cerebrospinal, the various transudates and exudates, the cystic fluids, the synovial and amniotic fluids.

SPECIFIC GRAVITY.—This may be determined with an accurate araëometer (see page 87). Our figures given in the following pages were determined gravimetrically.

DRIED CONSTITUENTS.—In a weighed glass dish with a ground-glass stopper are weighed or measured from 10 to 30 cc. of the fluid in question. This is evaporated over a water-bath and then in vacuo over sulphuric acid. It is then dried at about 110° to constant weight, no higher if urea or other fragile bodies, as is usually the case, are present.

PROTEIDS.—In all may occur serum albumin, serum globulin, in some fibrinogen; the albumoses are rare. True peptone is said never to occur, while the glyco-proteids and the phospho-proteids occur in some; *e.g.*, the cystic fluids.

For examination of the proteids it is first necessary to remove the organized structures. This may be done by sedimentation, centrifugalization, filtration through paper or Kieselguhr. If fibrin is present, it will be evident to the naked eye, and shown positively by the rapid solution of the clot in artificial gastric juice, and its glassy swelling on the addition of 0.1 per cent. hydrochloric acid.

ALBUMIN, GLOBULIN, FIBRINOGEN.—From 20 to 50 cc. are mixed with an equal amount of saturated $(NH_4)_2SO_4$ and allowed to stand one hour. The amount chosen should not contain more than 0.2 to 0.3 gm. of proteid for each precipitate. It is then filtered through a weighed filter, the precipitate washed with half-saturated $(NH_4)_2SO_4$ until the filtrate gives no cloud with acetic acid and K_4FeCN_6.

(*a*) The filtrate is boiled, acetic acid added until faintly acid, it then boiled again, and filtered through a weighed ashless filter. The precipitate is washed with hot water, then with alcohol, then with ether, and brought to a constant weight at 120° C. It is then ashed and the weight of this subtracted to give the weight of the albumin.

(*b*) The precipitate on the filter paper is heated to 110°, washed with hot water, then alcohol and ether, dried to constant weight, and its ash subtracted. This will equal the weight of the serum globulin and fibrinogen.

For the determination of both together, see page 614.

GLYCO-PROTEIDS and PHOSPHORUS-CONTAINING PROTEIDS. (1) *Mucin.*—In general 100 cc. of fluid, diluted if necessary with water, are precipitated with acetic acid, filtered, and the precipitate washed with water acidulated with acetic acid. The precipitate is then dissolved in weak alkaline water, and reprecipitated with acetic acid.

A. *Mucin. Mucoid.*—A part of this precipitate is boiled on the water-bath with dilute mineral acid (HCl) and filtered, and the filtrate tested for sugar. The reduction of the copper is not as ready as by pure glucose solutions, hence considerable boiling may be necessary, and the reduced copper seen only after the fluid is cold and the precipitate settled.

Mucin is a glyco-proteid of a stringy consistency, insoluble in acetic acid even in excess. *Mucoid* is similar in nature, but differs in some physical characteristics. A sharp line cannot be drawn (see page 211).

B. *Phosphorus-Containing Proteid.*—A part of the precipitate is examined for organic phosphorus. It is ashed, the ash dissolved in dilute HNO_3, heated to boiling, concentrated somewhat, and then ammonium molybdate added in excess. A yellow color, and then a yellow precipitate which forms most readily at 40° C.,

is evidence of phosphoric acid. Or the precipitate may be dissolved in HCl, made strongly alkaline with ammonia, and then magnesium mixture added. The white precipitate of NH_4MgPO_4 indicates phosphoric acid.

If the reaction for phosphorus be merely faint the test has no meaning, since all of the phosphates and lecithin cannot be washed from the mucin precipitate.

If organic phosphorus has been found, a part of the original precipitate is dissolved in NaOH, then HCl added, boiled to clear solution, supersaturated with ammonia and then precipitated with $AgNO_3$. A flocculent cloud indicates a nucleo-proteid, the silver salt of the nuclein base being precipitated. If none forms, the phosphorus body is a paranucleo-proteid.

Fat, Lecithin and Cholesterin.—To from 20 to 50 cc. of the fluid, weighed or measured, are added from 3 to 4 volumes of absolute alcohol. This is allowed to stand until the next day with repeated stirrings, then filtered, the precipitate washed with absolute alcohol, and the precipitate placed in the cylinder of a Soxhlet ether-extraction apparatus. The alcohol filtrate is neutralized and evaporated at 60° C. The residue is taken up with alcohol and ether and re-evaporated. The residue is then taken up with ether and placed in the flask of this same Soxhlet apparatus. The precipitate is then extracted for hours. The ether extract is evaporated, the residue taken up in water-free ether, the filtered solution evaporated in a weighed beaker, and dried in vacuo over sulphuric acid to constant weight. This will be the combined weight of the fat, lecithin, and cholesterin.

This residue is dissolved in alcohol, alcoholic KOH added, and it warmed on the water-bath for one hour, then evaporated to dryness. The fat is now soap and glycerin. To the residue is now added water (not too little) and this shaken out several times with equal volumes of ether.

The ethereal extract is distilled to small volume, then evaporated in a weighed beaker to dryness. The residue contains soap and cholesterin. The former may be washed out with cold alcohol (small portions) slightly acidulated with HCl. The cholesterin left is dried at 80° C. and weighed.

The alcohol washings with the soap are added to the water extract of the previous separation, which now contains all the lecithin-phosphorus. This fluid is evaporated, the residue ashed, and the phosphorus determined.

(Distearyllecithin contains 3.84 per cent. of phosphorus, dipalmityllecithin 4.12 per cent.)

Leucin. Tyrosin.—The fluid is examined as fresh as possible. All of the albumin is removed by heat and acetic acid, or by precipitation with from three to four volumes of alcohol, heating on the water-bath, cooling, and filtering. The alcohol is removed by evaporation. The filtrate is precipitated with neutral, then with basic, lead acetate, avoiding carefully any excess, and filtered. The lead is removed from the filtrate, with H_2S, the filtrate evaporated, and examined for crystals (see page 249).

Succinic Acid, $CH_2COOH.CH_2COOH$.—This acid occurs in many animal fluids in traces; sometimes in the fluid of hydrocephalus and hydrocele, much in echinococcus cysts, and in wool-fat. It is formed by the bacterial decomposition of proteids and sugar. It frequently occurs in acid milk in the intestine, in putrid pus, and in the alcoholic fermentation of sugar.

The fluid is freed of albumin by heat plus acetic acid. If it be urine which is tested, the albumin is first removed, the urine perfectly precipitated with baryta water, and the excess of this removed by H_2SO_4. The filtrate is evaporated to a residue, acidified with HCl, and extracted repeatedly with ether. The ether is evaporated off, the residue taken up with a small amount of water, and allowed to stand until crystallization. Or, the watery solution may be heated to boiling, nitric acid added drop by drop until it takes a slight yellow color, then evaporated. If no crystals form a portion of the residue is fused in a test-tube with ammonia

and zinc dust. If a match-stick wet with strong sulphuric acid is held at the mouth of the tube, the red color of pyrrol indicates succinic acid. In this test, however, hæmin and the indol derivatives must be excluded. These latter will give the reaction on heating alone. Hæmin on heating with zinc dust alone.

Lactic Acid, $C_3H_6O_3$.—Of the three modifications of lactic acid the inactive, or lactic acid of fermentation, occurs oftenest in the stomach and intestine of man. The dextrorotatory form, or sarcolactic acid, occurs in the muscles, blood, pericardial fluid, aqueous humor, and intestinal contents. It occurs also in the urine in acute yellow atrophy and phosphorus poisoning, liver cirrhosis after respiratory distress, severe exercise, and before death. It occurs in pathological transudates often in abundance, in the bones in osteomalacia, and in the sweat in puerperal fever. The lævorotatory form has never been found in the body.

The fluid to be examined is made, if necessary, faintly acid with dilute H_2SO_4, boiled and filtered to remove the albumin. Baryta water is added as long as a precipitate forms, and the excess of the barium removed with CO_2. The filtrate is evaporated to a thin syrup, without heating above 70°, in order to avoid the brown color. Absolute alcohol to about ten volumes or more in amount is then added slowly to the syrup, it is well stirred, allowed to stand for some time, then poured off. The residue is dissolved in a little water. The procedure is repeated with alcohol once more, continuing in the same way. The alcoholic solution is poured off, filtered, the alcohol distilled off to a thin syrup, and the residue digested on the water-bath at a moderate temperature to drive off the alcohol. It is then cooled. To the thin syrup is added an equal amount of dilute phosphoric acid; it is brought into a large flask and shaken out with a large amount of ether which gradually takes up the lactic acid. The ether must be frequently renewed. The united ether extracts are then filtered clear, the ether distilled off, the residue dissolved in water, boiled for some time with an excess of $ZnCO_3$, filtered, washed with hot water, evaporated to a small volume on the water-bath, and allowed to stand until the zinc salt of lactic acid crystallizes out. Alcohol is then added to the mother liquid, and it allowed to stand longer, and another mass of crystals is obtained. The zinc salt is dissolved in hot water and the zinc precipitated by H_2S. The filtrate is then evaporated to a syrup containing the lactic acid.

It is wholly untrustworthy to attempt to recognize lactic acid simply from its crystalline form or by Uffelmann's test alone.

Inosite $C_6H_6(OH)_6$.—This occurs in the urine of diabetics, in albuminuria, traces perhaps in each normal urine, but especially in polyuria, and in the echinococcus cysts.

The albumin is removed by heat, the phosphates precipitated by baryta water, the filtrate evaporated, and the creatinin allowed to crystallize out by boiling with from one to four volumes of alcohol. If a heavy precipitate results which sticks to the glass, the fluid is simply decanted, but if flocculent, it is filtered through a heated filter and then allowed to cool. The fluid then stands for twenty-four hours. If inosite is present, crystals will form which may be filtered out and washed with cold alcohol. The alcohol precipitate may be dissolved in boiling water, from three to four volumes of hot alcohol added, and the above procedure repeated to recover the inosite therein contained. If no crystals form, to the clear alcoholic filtrate is added little by little ether until a slight milky cloudiness results which does not disappear. This is then allowed to stand for twenty-four hours. All the inosite is precipitated as mother-of-pearl plates.

In case the urine is examined, it is first precipitated by baryta water and the filtrate, after heating, precipitated with PbAc, avoiding an excess. It is allowed to stand, is filtered, the precipitate washed, suspended in water, decomposed by H_2S, filtered, the filtrate evaporated. One then proceeds as above.

Inosite crystallizes in rhombohedral crystals which melt at 225° C., are soluble in water, 1 : 75, insoluble in alcohol or ether. It does not ferment, nor does it rotate the plane of polarization; it dissolves $Cu(OH)_2$ without reduction, is precipitated by PbAc, and does not give crystals with phenylhydrazine.

Scherer's Test.—A small amount of the precipitate of the crystals is evaporated with nitric acid on a platinum-foil almost to dryness. To the residue are added ammonia and one drop of $CaCl_2$ and the evaporation continued to dryness. A beautiful rose color results. The crystals must be pretty pure to give a positive test.

Seidel's Test.—This test is similar to the above with the exception that strontium acetate is used instead of calcium chloride, and a green color with a violet precipitate results. This test is positive if 0.3 mg. of inosite be present.

Allantoin, $(CO)_4(NH)_3NH_2$.—This body is found in the urine of the new-born child. A slight trace is said to occur in all normal urines, and especially those of pregnant women. It occurs in some ascitic fluids, in liver cirrhosis, and in certain ovarian cysts.

The albumin is removed by heat and acetic acid. The fluid is then precipitated with $HgNO_3$, the precipitate washed, suspended in water, decomposed with H_2S, and filtered. To the filtrate a little ammonia is added, and the whole evaporated to a small volume on the water-bath. The clear fluid is then precipitated with ammoniacal $AgNO_3$. (The precipitate is soluble in excess of ammonia, which must be avoided.) This is allowed to stand, the silver salt of allantoin is collected on the filter, washed, suspended in water, decomposed with H_2S, the filtrate evaporated, and allowed to crystallize.

The Loewy method is recommended for the urine. The faintly acid urine is precipitated with mercurous nitrate (which is dissolved in as little acid as possible plus some metallic mercury), filtered, the precipitate well washed, the filtrate is then precipitated with H_2S, and filtered, the filtrate warmed to drive off this gas, MgO then added, and the whole precipitated with $AgNO_3$. This precipitate is filtered, washed, suspended in water, and decomposed with H_2S while warm; the filtrate evaporated to dryness, the residue extracted with hot water, and when cold precipitated with $Hg(NO_3)_2$. The precipitate is well washed, decomposed with H_2S, the filtrate evaporated to a concentrated solution, whereupon the allantoin will crystallize out in glistening prisms, which are odorless, tasteless, soluble in 160 parts of cold water, and more in warm, insoluble in absolute alcohol or ether. For its identification the silver salts are studied, concentrated solutions being precipitated by ammoniacal $AgNO_3$. This precipitate is soluble in excess of ammonia. The white flocculent precipitate on standing becomes granular. If dried at 100° it gives an easy reduction of silver. The silver salt of the allantoin dried in vacuo gives on fusion 40.71 per cent. Ag.

Quantitative Analysis of Serous Fluids.—The method given by Thierfelder [1] is the one we use and of which we here give an outline. We have tried it on all sorts of fluids, and while each step is satisfactory, the routine, as a whole, is not, since fluids vary greatly in their composition, and the method hits none exactly. If one uses enough of some fluids to obtain a measurable amount of certain constituents, the amount of other constituents may be in such vast amounts as to render the whole impossible; determine these latter with a workable amount, and the former are an almost vanishing quantity. Hence it is better to determine each substance or group in a special portion. We give the outline of the analysis.

To from 20 to 50 cc. of the fluid measured or weighed, and freed from the

[1] Hoppe Seyler, Chemische Analyse, 1903.

formed elements by filtration, are added from three to four volumes of absolute alcohol. This is allowed to stand a few hours, filtered through a weighed filter, and washed a little with alcohol. This will be Filtrate I. The precipitate is then washed with boiling alcohol, into the same flask with ether, and then again with alcohol; these combined are Filtrate II. The precipitate is then washed with boiling water thoroughly, Filtrate III. The above precipitate will contain the proteid and a few salts, hæmoglobin if any be present, but not much of the other pigments. It is now washed once in alcohol and dried at 120° to constant weight, after the precipitate next to be mentioned has been added to it. It is then ashed and the weight of the ash subtracted from that of the precipitate, giving the weight of the *proteids*.

Filtrate I. is evaporated at a temperature not above 60° C. To this residue is then added Filtrate II. The residue and fluid are well mixed and the fluid decanted through a dried and weighed paper. It is then washed repeatedly with absolute alcohol, then with ether, and decanted each time, but none of the precipitate is allowed to get onto the paper (B 3). Onto the residue is now poured Filtrate III., the residue well mixed, and then all filtered through the same filter-paper, but into another flask from that which has received the above-mentioned decanted fluids. The precipitate is now brought on the paper and washed with water. This precipitate is dried and added to the above-mentioned proteid precipitate, since it contains that amount of proteid which was lost in the first precipitation. The watery extract of the above is evaporated in a small weighed porcelain dish in a water-bath, dried at 110° to 115° C. to constant weight, and weighed.

B. 2. It is burned at a moderate heat, ashed, and the weight of the ash determined.

B. 3. The alcohol-ether extract is evaporated on the water-bath at a temperature not above 60° C., dried in vacuo over H_2SO_4. To the residue is added ether, and filtered into a flask through a small paper, washing repeatedly with ether. This will contain the urea, sugar, soaps, sodium chloride, fat, lecithin, cholesterin, and the cholesterin ester.

B. 3 *a* is the residue of the above. This is washed from the beaker and paper into a small weighed porcelain dish, evaporated, dried at a temperature from 110° to 115°, fused at a moderate heat, and weighed.

B. 3 *b*. The ethereal extract is treated as on page 611. It contains fat, cholesterin, lecithin, cholin, etc.

CEREBROSPINAL FLUID

The *normal amount* varies greatly; often from 5 to 10 cc. or more may be obtained. The fluid is relatively most abundant in the first years of life. It is pathologically increased in certain infectious diseases, in meningitis, and always in hydrocephalus and in general paralysis of the insane. Coriat found it increased in alcoholic cases (10 to 100 cc.), in dementia præcox (even 50 cc.), and in general paralysis, sometimes over 100 cc. In senile cases it was even 60 cc.

In *color* the fluid is either absolutely limpid or has a slight yellowish color due to lutein, the pigment of blood-serum. In subdural hemorrhage the fluid may be red, in jaundice a greenish-yellow, while the presence of pus will of course give it an opaque yellow color. It is also stained by certain drugs, as, for instance, methylene blue.

In *reaction* it is normally alkaline, but rapidly becomes acid postmortem. Its reaction varies much, and it is said sometimes even to be acid during life, due to lactic acid fermentation. Post-mortem in even ten minutes it has been found acid, for instance in one case after epi-

leptiform convulsions, in which case the (inactive) lactic acid formed in the brain was said to have been present. Its reaction depends directly upon the reaction of the brain-tissue.

The *specific gravity*, Coriat states, is from 1007 to 1010 normally (Halliburton, 1006 to 1008). In various diseases this varies much, and nothing specific has been determined. In general paralysis 1009 to 1012 are common figures; in hydrocephalus 1008 to 1009, thus within the normal limits.

In the cerebrospinal fluid is found a *reducing body*, 0.04 to 0.05 per cent., the nature of which has been much disputed. Halliburton claims that this is similar to pyrocatechin, that it is not sugar, is always present, and is increased by repeated tappings. This reducing body reduces copper but not bismuth, does not ferment, is optically inactive, and gives no osazon with phenylhydrazine. Mott and Halliburton found this body absent in twelve of fourteen cases of general paresis; it is absent in tuberculous, and especially epidemic, cerebrospinal meningitis. Others claim that glucose is present in hydrocephalus (Cavazzini); in diabetes (Schaefer), in which case 0.32 to 0.35 per cent. is said to have been found; in grave pneumonia it is increased. Coriat thinks that the body is glucose, or, at least, is not pyrocatechin.

Urea is present from 0.01 to 0.05 per cent., and has no pathological significance. Much is present in hydrocephalus, in which condition the fluid is almost normal; much in nephritis, even 0.45 per cent., and considerable in arteriosclerosis.[2]

The *proteids* found are globulin, nucleo-proteid, and protalbumose. The total proteid according to Quincke is from 0.2 to 0.5, Ricker 0.5 to 1, and Gumprecht 0.25 per litre. Halliburton found that of thirteen cases, in all globulin was present; in six cases albumose also, while in three, two of which were clearly cases of inflammation, albumin also. No fibrinogen is ever found normally. (To detect this a little blood-serum is added to the cerebrospinal fluid, and its presence is evinced by fibrin formation.) Serum albumin is said to be normally absent. In meningocele albumose and peptone have been found.

In general paresis the total solids are even 2.39 p. m.; the proteids are considerably increased. There is some increase in hydrocephalus, in inflammatory conditions, in cases with stasis due to brain tumor (2 to 4 p. m.), after repeated tappings, in apoplexy, and in meningitis (as a rule, 2 to 3 p. m.; but if purulent 7 to 9 p. m.).

Mott and Halliburton recommend the following quantitative method: The fluid is made acid with acetic acid, two volumes of absolute alcohol are added, it is then boiled, filtered, the precipitate dried at 110° C., and weighed.

[2] See Widal and Froin, Gaz. des Hôp., No. 122, 1904.

In eight cases of general paresis the average percentage was 0.239. In two of spina bifida 0.088 per cent. In general paresis proteoses and peptones were absent and the proteid was chiefly globulin with a little nucleoproteid.

This latter is determined in one litre of the fluid to which alcohol has been added, and the precipitate digested in water. If the undissolved residue is found to contain a high percentage of phosphorus, indicating nuclein, the residue is washed with 0.2 per cent. HCl, heated on the water-bath at 100° C. with fuming HNO_3 and a small amount of H_2SO_4 and $KClO_3$. The residue is dissolved in HNO_3 and ammonium molybdate added; a yellowish crystalline precipitate results.

A very good idea of the amount of proteid present may be gained by the heat-acetic-acid test. A normal fluid remains clear on standing some hours. If it be boiled no cloud at all results, but on adding a drop of dilute acid a very faint white opalescence appears, which will separate in fine flocculi. It is easy to recognize a denser cloud than normal.

Saline constituents present nothing interesting; they resemble those of other serous fluids.

The *toxicity* of the fluid has been found increased in general paresis, also after epileptic seizures. Its poisonous qualities are due to cholin and other products of nerve degeneration.

Cholin is a decomposition product of lecithin, the chief component of the myelin sheaths, and is found where there is nerve disintegration. It is a body which is soluble in water and alcohol, insoluble in ether, is precipitated by $PtCl_6$ as polymorphous crystals, which, however, if recrystallized from warmed 15 per cent. alcohol are regular octahedra. These crystals are insoluble in alcohol and ether, but soluble in water.

The careful technique given by Coriat is as follows: The proteids are first precipitated by 95 per cent. alcohol in excess, the filtrate evaporated over the water-bath at 40° to dryness, extracted with absolute alcohol, filtered again and evaporated to dryness. This process is repeated several times, the temperature always being kept low. All traces of proteid and potassium salts are thus removed. The final residue after extraction with absolute alcohol is a syrup of a light color, which is divided into two parts. The first is dissolved in distilled water, and the second in 15 per cent. alcohol. The watery solution is tested for proteid by the biuret, Millon, and other proteid reactions, which must all be negative; for cholin by the ordinary reactions for alkaloid (phosphotungstic and phosphomolybdic acids, *et al.*), which must all be positive. To the alcohol solution are then added four drops of 4 per cent. $PtCl_6$, and this is evaporated in a watch-glass over $CaCl_2$. For a positive test tannic acid should give no precipitate (thus neurin is excluded); phosphotungstic acid a white precipitate, phosphomolybdic a yellow, also $PtCl_6$ and $AuCl_6$, and Lugol's a brown precipitate. On evaporating the 15 per cent. alcohol solution, large yellow octahedral crystals must be formed, easily soluble in water (therefore not neurin). Their size, solubility in water, and the fact that the watery solution gave the alkaloid reaction, excludes potassium. These crystals, if in a sufficient amount, may be dried and the platinum determined, which should be 34.8 per cent.

In the same hydrolysis with lecithin are formed the glycero-phosphoric acids and stearic acid. The latter unites with the glycerol radicals to form the neutral fats upon which the Marchi stain depends.

The cholin is eliminated in the cerebrospinal fluid and the blood. Glycerophosphoric acid is eliminated in the urine.

The presence of cholin indicates nerve disintegration. It has been found, however, in a wide variety of nervous disturbances, general paresis, combined sclerosis, insular sclerosis, alcoholic neuritis, beriberi, senile dementia, delirium tremens, *et al.*, roughly in amount parallel to that of proteid present, both being a measure of nerve-tissue disintegration. Mott considers that it cannot be used to separate the organic from the functional disturbances unless the organic disturbance be active at the time the fluid is examined. Although it occurs in such a variety of cases, its most constant occurrence is in general paresis, in which disease Coriat found it present in all of fourteen cases, yet with no relation between amount and anatomical findings.

We add a table of a few analyses we have recently made, calling particular attention to the high solid content in stasis (due to brain tumor), and to the difference between the ventricular and spinal fluids in a case of hydrocephalus, a difference which we had noted in two previous cases.

The cerebrospinal fluids will not keep, in fact are full of bacteria in a few hours; hence must be used while fresh.

As regards cholin our experience is limited, but we fail to find the demonstration of octahedral crystals so very easy.

CEREBROSPINAL AND VENTRICULAR FLUIDS

No.	Case.	Amount.	Sp gr. (grav.)	Solids, per cent.	Proteids, per cent.	Salts and Extractives soluble in H_2O; insoluble in Alcohol.	Total Ash, per cent.	Extractives and Salts soluble in Alcohol; Urea, Sugar, NaCl, etc.	Total Ash, per cent.	Soaps, Glycerin, } fat. Cholesterin, Cholin, etc., per cent.
1	Normal child......	13	1007.4	......	......	......	......	......	......	.....
2	Normal child......	22	1008.3	......	......	......	......	......	......	......
3	Hydrocephalus:									
	Cord	......	1002.	......	......	......	......	......	......	......
	Brain (fluid from)	......	1006.2	0.96	0.0991	0.2703	0.507	0.2937	......	......
4	Hernia of brain									
	(tumor)..........	50	......	......	0.170	0.62	0.492	0.374	0.2092	0.023
	Later	630	1006.9	2.5132	0.1112	0.5964	0.5166	......	......	0.016
	Later.............	450	1007.7	......	......	......	......	......	......	......
	(Ventricular fluids.)									
5	Tumor of brain....	200	1011.6	......	.....	......	......	......	.....	......
	(Hernia.)									
6	Gunshot wound;									
	head.............	25	1009.2	......	2.664	0.7839	0.68	0.1988	......	......
7	Cerebrospinal									
	meningitis.......	100	1007.	......	......	......	......	......	......	......
8	Streptococcus									
	meningitis.......	25	1009.2	......	0.1628	......	......	......	......	......
9	Tuberculous meningitis									
	ingitis..........	......	1018.8	......	0.066	0.5629	0.4712	0.4112	0.2445	0.0185
10	Pneumonia; meningeal symptoms..	10	1006.	......	......	......	.....	......	......	......
11	Paresis?..........	30	1008.	......	0.0597	......	......	......	......	......

Cytodiagnosis of Cerebrospinal Fluid.—A good deal of attention has recently been directed to this subject, especially by the French for the diagnosis of general paresis, tabes dorsalis, and lues. Widal and others have shown that in these conditions, really diseases with chronic posterior meningitis and small round-cell infiltration of the meninges, there is a lymphocytosis which fails in functional cases and in conditions in which the meninges are not infiltrated. In purulent infections one will find a predominance of pus-cells. In tuberculosis there does not seem to be the same constancy of a lymphocytosis as in tuberculosis of the pleura, although if lues can be ruled out a lymphocytosis is said to suggest tuberculosis (when the clinical symptoms point to meningitis). But it may be due also to any other long-continued irritation, as lues, uræmia, tetanus, and mechanically by tumors.[3] Fischer considers in general paresis the chronicity to be very important, only the acute cases showing a lymphocytosis, the chronic cases none.

To get a fairly correct idea of the number of cells in the fluid the technic must be at least uniform. The method of Marie's clinic is as follows: Three cubic centimetres of the fluid are centrifugalized for fifteen minutes in 15 cc. tubes with very sharp points. As much of the fluid as possible is then decanted, and the edge of the tube blotted. With a very fine pipette is then taken up the very small drop, all that can be collected from the end of the still inverted centrifuge tube. This is then spread on a slide as a round area of uniform size about 7 mm. in diameter. This is stained with any polychrome methylene blue mixture and examined with the 400 magnification. A few lymphocytes are normal. An increase can merely be estimated by a comparison based on the uniform technic.

One point must be emphasized, since we know of three mistakes made by neglecting it. Red cells are not rare in the fluid, from slight hemorrhage due to the needle. Some of them will take a deep blue color, and with the magnification used will be counted as lymphocytes unless one be on his guard.

Using technic similar to the above Widal considers 4 or 5 lymphocytes per field of the 400 magnification as the maximum normal; as a rule, none are found.

In cases with stasis (hernia cerebri) occur large cells full of large fat-like granules.

Attempts have been made to count the cells directly. Kramer[4] found in general paresis 6 to 145 per cubic millimetre, in dementia præcox 0 to 2, and in other cases 0 or 1 cell.

TRANSUDATES AND EXUDATES

Although the pathologists will not agree, the clinical chemist may call a transudate a fluid which is not the result of an inflammation,

[3] Schlesinger, Deut. med. Woch., 1904, No. 28.
[4] Am. Jour. Insan., vol. lx. p. 107.

but more of a filtration; while an exudate is the result of an inflammation. The transudates resemble lymph, contain few formed elements and almost no fibrin. Their proteids are serum albumin, serum globulin, a little fibrinogen, yet not enough to coagulate spontaneously, although they will if blood be added. The exudates are richer in formed elements, coagulate spontaneously, contain the so-called nucleo-albumin and a mucoid substance. The specific gravity varies as the amount of albumin. Some claim that that of the transudates varies from 1015 to 1018, and if over 1018 the fluid is an exudate. This rule is practically true, and yet often fails. The list of extractives which may be present is, urea, glucose, creatinin, uric acid, lactic acid, inosite, succinic acid, allantoin; pathologically occur leucin, tyrosin, bile acids and pigments, fat, lecithin, and cholesterin.

This "nucleo-albumin," or better "euglobulin," is valuable in distinguishing these two classes of fluids. If a few drops of acetic acid be added to a clear exudate, a cloud of varying depth, but usually quite dense, will form. It is rather soluble in excess of the acid. The cloud in transudates is very much lighter.

Peritoneal Fluid.—In cachexia and hydræmia this fluid is slightly colored, of a milky opalescence, does not clot spontaneously, has a specific gravity of from 1005 to 1015, and almost no cells.

In chronic passive congestion the specific gravity is usually lower than 1020. Sometimes it contains 35 gms. per litre of proteid. In cases of cancer of the peritoneum the fluid is turbid with cells, of a dirty grayish appearance, a high specific gravity, and often clots spontaneously. The serous fluid present in inflammations is of a straw or lemon-yellow color, somewhat cloudy from the formed elements, coagulates spontaneously; proteid 30 gms. or more per litre, and a specific gravity of 1030 or above. Mucoid substance is perhaps always present.

This is proven by removing the albumin by heat and then by precipitating the filtrate by alcohol. A precipitate is formed from which one can split off a reducing body.

In the ascitic fluid may also be determined urea, uric acid, allantoin, xanthin, creatinin, cholesterin, and sugar.

The fluids we have examined had a specific gravity varied from 1005.5 to 1019.8, the solids from 1.3 to 4.5 gms. per litre, globulin, 40 to 50 per cent. None were very acute cases.

Pleural Fluids.—Physiologically, there is not enough present to be analyzed. The pathological fluid may vary from serous to sero-purulent to purulent or hemorrhagic. In hydrothorax the specific gravity is lower than 1015 as a rule, the albumin from 10 to 30 gms. and the fibrinogen hardly 0.1 per litre. In pleurisy the exudate has a specific

gravity above 1020 as a rule, albumin 30 to 65, and the fibrinogen 1 per litre.

In nine recent cases the specific gravity varied from 1012.2 to 1025.2, and the solids from 3.12 to 7.926 per cent., the higher being in the acuter cases. The total proteid was 2.837 to 6.529 per cent., of which the serum globulin was from 39 to 64 per cent. The acuter the cases the more the globulin. The acetic acid precipitate was markedly more in the acute inflammatory cases than in the transudates. It is interesting what a little difference the clotting makes. In a case of acute tuberculous pleurisy, before clotting the specific gravity was 1022.1 and the globulin 2.875 per cent.; after clotting (densely), the figures were 1021.7 and 2.376 respectively. To clear these fluids with the centrifuge is better than with Kieselguhr, since the extractions found are lower in the latter case.

The cytodiagnosis of the pleural and ascitic fluids has been a fertile subject recently, all work directed to the one point, the possibility of a diagnosis of tuberculous pleurisy or peritonitis based on a lymphocytosis. Other organisms cause a migration of the polymorphonuclear cells. Concerning the tubercle bacillus there are some who believe that the lymphocytosis is an active migration of the small mononuclear blood-cells; others that these cells are really derived from the endothelial lining of the serosa.[5] It is true that in some cases of so-called proliferative pleurisy or peritonitis there are found a great many free endothelial cells and large groups in mulberry-like masses in the fluid. It is also true that these endothelium cells undergo degenerative changes, the result of which is a cell similar to a lymphocyte; but in tuberculous pleurisy the cells are chiefly typical lymphocytes and the stages of condensation of larger cells not so evident. In Fig. 123 we have drawn some of the cells from a chronic pleurisy, showing their transitions from very large flat to small compact cells. The lymphocytes in the picture seem different.

These fluids are best examined very fresh, the cells centrifugalized before clotting occurs. Smears are made, and stained with a polychrome-methylene-blue-eosin mixture. That recommended by Musgrave is Wright's mixture diluted with methyl alcohol 3 : 1. This stain is left on one-half minute, then diluted with water for three minutes (see page 425).

In hydrothorax the cells are few and mainly endothelial; even in the serous stage of pneumococcus and streptococcus pleurisy there is a great preponderance of polymorphonuclears; in tuberculous inflammations the lymphocytes are the predominating cell, since the infection is weak and long-standing. This is as far as most observers go. Others go farther. Barège, for example, says that in the fluid of cardiac and Bright's disease, if the fluid contains many endothelial cells, few small mononuclears, and fewest polymorphonuclears, the origin is mechanical; a slight increase of the last cells means congestion of the lungs, a considerable polynucleosis means lung infarct. The work

[5] See Patella, Deut. Arch. f. klin. Med., April 17, 1902.

of 1903 was in favor of cytodiagnosis, but then the pendulum swung, and now workers are sceptical.[6]

A study of cases from this clinic was made by Bunting.[7]

The exceptions to such rules are too numerous. Some find lymphocytosis in transudates, in pneumococcus, influenzal, and staphylococcus infections; and polynucleosis when strictly there is no inflammation, as in infarcts, sunstroke, cancer. Some called the findings "relative," others said "problematical." We use cytodiagnosis very little now,

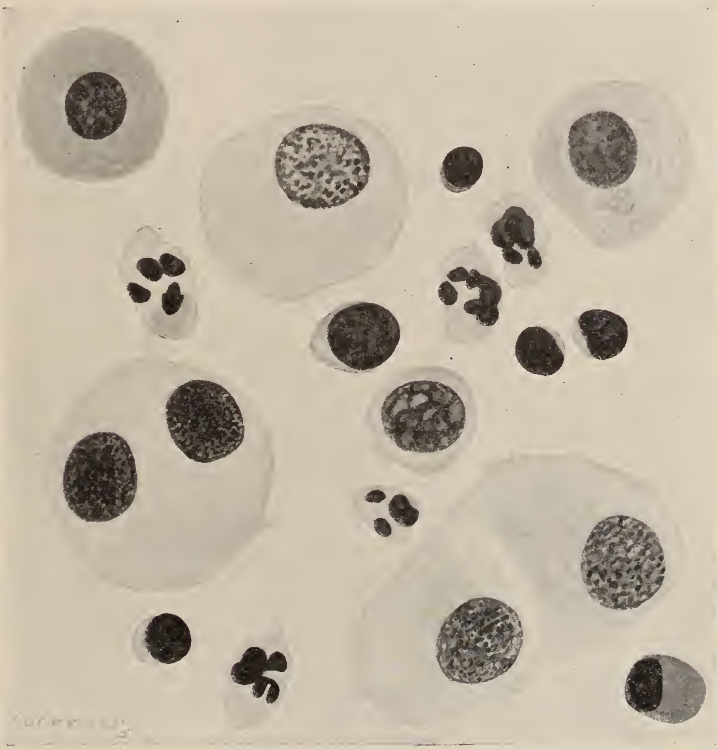


FIG. 123.—Cells from a pleural exudate, showing the transitions from large to small cells (lymphocytes). × 900.

since it is easier to find the organisms themselves, and then we have something certain.

In some cases Mastzellen are greatly increased. In tuberculous pleurisy there is a slight eosinophilia (2 to 5 per cent.), in acute pleurisy sometimes a true (of 10 to 74 per cent.) which means a good prognosis.

The possibility is always present of diagnosing cancer of the pleura

[6] See especially Miller, Am. Med., November 12, 1904.
[7] J. H. H. Bull., July, 1903.

or peritoneum by the cells of the fluid,[8] and especially by the small mass sometimes found sticking to the end of the needle.

Inoscopy is the method proposed by Jousset[9] for isolating the tubercle bacilli from exudates. The fluids were allowed to coagulate spontaneously, or a little horse serum added which produced this result. The clot is digested, the resulting fluid centrifugalized, and smears made and stained for tubercle bacilli. The clot will have caught most of the organisms. It is first pressed out, then torn into fragments and mixed with a fluid consisting of pepsin, 1 to 2 gms., glycerin, 10 cc.; HCl 40 per cent., 15 cc.; NaF, 3 gms.; water, 1000 cc. Jousset claimed that digestion took but three or four hours. He obtained astonishingly good results, so good that his critics hint he mistook masses of hæmoglobin or fuchsin and scratches on the glass, etc., for tubercle bacilli (Körmöczi and Jassinger).

The fluid may be centrifugalized while very fresh, or injected into a guinea-pig. We have not had good success with inoscopy.

Pericardial Fluid.—This fluid normally is of a lemon-yellow color, slightly viscid, and seems to contain more fibrin than other physiological fluids. The solids are from 37.5 to 44.9 gms. per litre; albumin, 22.8 to 24.7; soluble salts, from 8 to 9; insoluble salts, 0.15; extractives, 2 per litre.

The fluid of a recent case had a specific gravity of 1020.4; solids, 5.8 per cent.; total proteid, 3.91, and globulin only 15.2 per cent. of this.

Synovial Membrane.—The fluid is alkaline, thick, sticky, viscid, yellowish in color, cloudy often from the cell detritus, or clear; contains albumin, salts, and a body which is physically like mucin, but is not, since no reducing body can be split off. Neither is it nucleo-albumin. Salkowski has given it the name "synovin."

The fluid from a recent case of rheumatism, and which clotted firmly, had a total proteid content of 4.3 per cent.; water-soluble extractives, 1.07 (ash, 0.606) per cent.; alcohol-ether-soluble extractives, 0.076 (ash, 0.046) per cent.; fat fraction, 0.35 per cent.

Chylous Fluids.—Fluids which are milky in appearance have always attracted considerable attention. Sometimes a fluid may be truly chylous, in which case from 3.86 to 10.3 per litre of fat are often obtained, and in Minkowski's case from 17 to 43 per litre. Such fluids clear on shaking out with ether. In other cases, however, the fluid does not contain nearly enough fat to explain the turbidity. By some lecithin is supposed to explain the cloudiness, but other fluids with much lecithin are clear, and some milky fluids have very little (Christen). Others consider that globulin explains it, or casein or seromucoid. The most recent explanation is that it is a compound of globulin and lecithin, whether in combination or not is uncertain. Bernert, who examined one case with exceeding care, sums the matter up as follows: There

[8] Steiner, J. H. H. Bull., October, 1901.
[9] La Sem. Méd., No. 3, 1903.

are cases in which the milkiness is not due to fat alone, but to albumin of the globulin group from which large amounts of lecithin can be extracted by hot alcohol. The fat content is low, and resembles that of the so-called fatty degeneration of the epithelial cells. Quincke first showed that albumin also in fine granules could give a milky appearance.

The color of such fluids is white or yellowish-white, greenish or reddish, opalescent in thin layers. Some fluids become more milky on cooling. In some cases a perfectly clear fluid on the first tapping becomes progressively more milky on the subsequent. On standing the fluid will sometimes deposit a sediment and have a well-marked cream on the surface. Filtering or centrifugalizing does not clear it. The specific gravity varies from 1010 to 1014. In one case it was 1061, in another 1081, in which cases much pus must have been present. Their reaction is alkaline, and, strange to say, there is no odor. In the cases that we have examined this has been a marked feature. They are very resistant against decomposition, and the fluids could remain in the laboratory for weeks without apparent change. The sediment is slight, consisting of epithelial cells, all degenerated with fatty globules, and globules which do not take the stains of fat.

In general there are two classes of cases,—those very milky, the "chylous," and the "chyliform," which are only very opalescent. In this case we used the terms only as descriptive without implying that chyle was or was not present.

The former occur when chyle is present, as in the traumatic cases; in others the fat may best be explained by the fatty globules freed from the fatty degenerated epithelium cells. More cases it is hard to explain. Our best case was one of tuberculosis of the peritoneum. In Tabora's case of peritonitis carcinomatosa the fat was 1.2 per cent.; sugar, 0.864 per cent.

In our case of markedly chylous ascites the specific gravity was 1013.3; proteid, 5.114 gms. per litre; globulin, 73 per cent. of this. The fat-cholesterin-lecithin-fraction 1.469 per cent.

The opalescent fluids occur in a great variety of conditions, and are often found at autopsy; cachexias, anæmias, heart cases, etc.; Naunyn stated that the cause in many cases is amyloid degeneration of the blood-vessels of the serosa. The reason suggested for cases in heart-failure is the stasis in the thoracic duct; in other cases stasis due to pressure of tumors on duct.

A chyliform ascitic fluid from a case of uræmia had a specific gravity of 1005.5, and solids 1.2988 per cent.

OVARIAN CYSTS

Colloid is not one substance, the name being based on the physical properties of the contents of various cysts and organs. They are gelatinous, insoluble in water and acetic acid, soluble in alkali. From some may be split off a reducing body, but their composition varies much.

Pseudomucin (Metalbumin).—This body occurs in many ovarian cyst contents which are very viscid and slimy. Alcohol gives a thready precipitate resembling wood-pulp, which can be wound around the rod. It is not precipitated by heat nor by acetic acid. The precipitate formed by alcohol is ground fine under alcohol, and then freed from alcohol by means of ether, and dissolved in water. It is then reprecipitated with alcohol. A light white powder is obtained which is soluble in water to an opalescent mucoid solution, which is not well precipitated

by acetic acid. When boiled with HCl an abundant reducing body is split off, which reduces copper very easily.

Paramucin.—This is a substance present in certain ovarian cysts, also in the ascitic fluid providing the ovarian cyst has already ruptured into the abdomen. It is firm, glistening, with the consistency of gelatin, soluble in dilute mineral acid, shrinks in acidulated alcohol, or in alcohol and ether, and can be reduced to a fine white powder. Its characteristics are, its insolubility in water, the fact that it swells in alkali dissolving in excess, is precipitated by acetic acid and soluble in excess, and, especially, it will reduce copper salts without preliminary boiling with acid.

Serous cysts (dilatation of Graafian follicles) contain a perfectly clear serous fluid, watery, which foams easily, is of amber color, of a

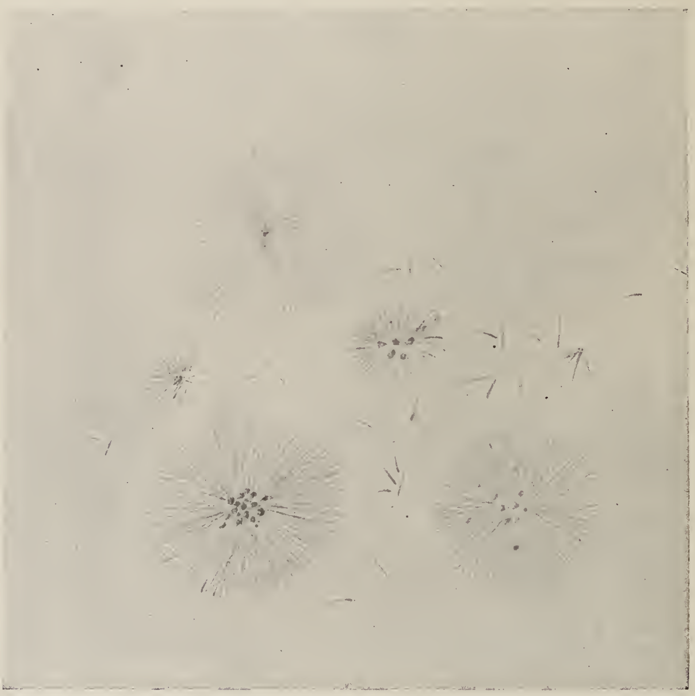


Fig. 124.—Fatty acid crystals from the contents of an ovarian cyst. × 400.

specific gravity from 1005 to 1022 (usually 1005 to 1014), with solids from 10 to 40 per litre and all the other constituents of serous fluids.

In two recent cases the specific gravity was 1022 in one case, 1016 in another; they contained a great deal of albumin. Heat alone caused but a faint cloud, but one drop of acid made the fluid perfectly solid. Both serum globulin and serum albumin were present; in these little or no euglobulin.

Proliferating Cysts from Pfluger's Tubules.—The contents of these are various. Some contain "colloid," and on boiling with acid give a reducing body. From the colloid ovarian cysts the fatty crystals (solu-

ble on warming) may be watched to crystallize out singly and in rosettes (see Fig. 124).

Another group of cysts contain a viscid fluid, very stringy, which varies much in consistency according to the amount of serous fluid present. It is of a brownish or dark greenish-brown color.

The specific gravity of the contents in three recent cases was 1025 to 1030.2; the solids were 9.7 and 9.3 per cent.; the alcohol precipitate, 6.9 and 8.5 per cent.

Some, however, contain a thin watery fluid, of a bluish-white opalescent color which may, however, be yellowish, yellowish-brown, or greenish, according to the amount of blood present.

We give a few examples of the contents of such cysts which we have recently seen.

1. Fluid quite opalescent; specific gravity, 1004.3; solids, 2.837 per cent. Alcohol precipitate 1.98 per cent. resembles macerated filter paper, is not stringy, can be reduced to a fine white powder, difficultly soluble in water to an opalescent fluid. Watery extractives 0.524 (ash, 0.388) per cent. Alcohol-ether soluble extractives, 0.2056 (ash, 0.108) per cent.; fat, cholesterin, etc., 0.96 per cent.

2. Fluid reddish-yellow, considerable sediment of small epithelial and some

FIG. 125.—Cholesterin crystals. × 400.

large epithelial cells with coarse refractile granules; filters clear. Specific gravity, 1008.04; solids, 2.32 per cent. Alcohol precipitate similar to above.

3. Bluish opalescence; specific gravity, 1007.3.

Of some of the multilocular cysts the contents are thick, not especially viscid, but a suspension of glistening masses of cholesterin crystals and of a yellow-red or brown color, depending on the blood pigment.

Of a recent case the figures were, specific gravity, 1025.9. The alcohol precipitate, 10.56 per cent., contained no reducing body. Half saturation of the original filtered fluid with $(NH_4)_2SO_4$ gave a precipitate 0.692 per cent. (globulin?); albumin? 0.604 per cent. Extractives, soluble in water, 1.46 (ash, 0.266) per cent.; alcohol-soluble extractives, 0.56 (ash, 0.44) per cent. Microscopically a great amount of detritus in the sediment, with very large cells (epithelial) full of glistening granules, cholesterin crystals, and fat needles.

In another similar case the specific gravity of the fluid was 1030.6.

The sediments contain much detritus, red blood-cells, leucocytes, large epithelial cells, single and in groups, filled with granules like fat,

large masses of fatty granules, cholesterin crystals, and colloid granules which are large, circular, strongly refractive bodies.

In the case of a dermoid, the contents of which contained much paramucin, serum globulin and albumin could also be demonstrated. Water content of the jelly, 92.2 per cent. The alcohol precipitate in one case was 3.3 per cent. Water-soluble extractives, 0.4 (ash, 0.27) per cent. Alcohol-ether extractives, 0.25 (ash, 0.16) per cent.

Tubo-ovarian Cysts.—The contents of these are watery, thin, serous, and contain no pseudomucin.

Parovarian Cysts.—These contain a thin watery fluid of a very pale yellow or colorless or slightly opalescent appearance. Specific gravity,

FIG. 126.—Sodium biurate crystals from a tophus. × 400.

1002 to 1009; solids from 10 to 20 per litre; no pseudomucin. Albumin may fail entirely or be only slight in amount. It consists, therefore, of water and extractives.

In a recent case, age twenty years, the cyst contained about 2 litres of very clear watery fluid with very slight opalescence; specific gravity, 1007.8; only the faintest precipitate with alcohol or ammonium sulphate; chlorides, 0.45 per cent. (as NaCl). Microscopically there were very few epithelial cells, round, granular, with a round nucleus.

Intraligamentous Cysts.—The contents of these are yellow, yellowish-green, or brownish. They contain little or no pseudomucin; specific gravity, 1032 to 1036; solids, 90 to 100 per litre, and the proteids of the blood plasma.

Hydrocele.—The contents are of a high color, clear or dark yellow, or greenish; specific gravity, 1014 to 1026; the solids on an average of 60 per litre. The fluid sometimes coagulates spontaneously. Leucocytes are always present, sometimes cholesterin crystals.

For illustration, in one case the specific gravity was 1014.7; solids, 6.329 per cent.; total albumin, 5.92 per cent., of which 45 per cent. was globulin; water-

soluble extractives, 0.7504 (ash, 0.462) per cent.; alcohol-ether extractives, 0.452 (ash, 0.1726) per cent.; fat fraction, 0 1864 per cent.

Spermatocele.—The fluid of this is colorless, watery, slightly milky; specific gravity, 1006 to 1010; average solids, 13 per litre; the proteid content slight, and contains cell detritus, fat granules, and spermatozoa.

Tophus.—The tophi of gout, so important in diagnosis, can only be distinguished from small sebaceous cysts, small cartilaginous tumors, etc., by the microscopic examination of their contents. A little is mixed with water and found to be an amorphous paste, with a great many needles of sodium biurate. (See Fig. 126.) An amorphous sebaceous matter with many fatty and cholesterin crystals is found in sebaceous cysts.

The masses of **urea crystals**, the "urea frost," which appear on the skin of the face in rare cases of nephritis just before death (there have been but five cases in this clinic) may be tested by the method given on page 109. This is a most interesting phenomenon; the circulation in the skin when it occurs is so poor that it is very hard to believe the source is immediately the blood.

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